

EFFECT OF ANOXIC ANOXIA ON THE HUMORALLY STIMULATED PANCREATIC SECRETION

BY

KAARLO J. V. HARTIALA

*Academic dissertation to be presented with the consent of the medical faculty of the
University of Helsinki for public examination in auditorium XII
on September 8, 1951 at 12 o'clock noon.*

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KIRJAPAINO O.Y. SANA

To my wife

Preface

The present studies were began in Helsinki in 1947 at the Physiological Institute, Univ. of Helsinki. It gives me great pleasure to express my sincere thanks to Professor YRJÖ REENPÄÄ for making available the facilities of his institute and his personal interest for the advancement of this investigation. This interest has been untiring and has followed the work up to its final stages.

The low pressure chamber used in the study was placed at my disposal by Doctor T. Y. ROSCHIER; Chief Physician of the Tilkka Military Hospital in Helsinki. It is not the first time I have had the opportunity to rely on the kindness of Doctor Roschier. For his kindness I am very grateful.

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To the volunteer subjects participating in the study I wish to thank for their tireless cooperation and for the pleasant way they carried on the most unpleasant part of the investigation.

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Helsinki, 12 March. 1951.

K. H.

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Introduction

The ill effects of rarified air on living organisms have been known for many hundreds of years. The origin of the oldest known report on the deleterious effect of low atmospheric pressure has been ascribed to ARISTOTLE who claimed that men could not live on the top of Mount Olympus without breathing through a wet sponge.

Scientific approach to the problems relative to the various phenomena connected with the functions of organism exposed to low oxygen environment could begin only after oxygen had been discovered and isolated. The invention of the barometer and the pneumatic chamber provided further essential tools for investigation.

It remained, however, for CLISSOLD in 1823, some fifty years after the real discovery of oxygen by LAVOSIER (1777), to attribute mountain sickness to reduced oxygen in air. The pioneer worker in the field, PAUL BERT (1878) established the modern concept of the effect of decreased partial pressure of oxygen as the causative factor in various physiological effects associated with high altitude conditions.

Since then physiological changes in the respiratory and circulatory systems of an organism exposed to lack of oxygen have been determined. It has become evident that other organ systems also manifest changes in these conditions.

The modern science of aviation has been a great stimulus for research in this field and as a result of all the academic interest, the practical necessity and the urge for better knowledge, a vast amount of literature has accumulated on subject. Old concepts have been corrected and new fields of argument have been both opened and occupied.

The literature has been reviewed several times. It appears to be of particular interest that, although gastrointestinal disorders have been regarded as one of the symptoms of mountain sickness and that some of the digestive functions have received attention, relatively little is known about the function of the most important enzyme producer of the digestive tract, the pancreas, under anoxic conditions. The most recent monograph published by VAN LIERE in 1942 has no reports on the effects of anoxia on the external pancreatic secretion.

The aim of this paper is to furnish some additional information relative to the secretory function of the pancreas under the conditions of anoxic anoxia.

Chapter I Approach to the problem

Definitions and descriptions

Although anoxia literally means »without oxygen» the term is generally used to designate those conditions also where the tissues have only a partial lack for oxygen. The more correct term would be hypoxia. In fact this term is also used in the literature and the suggestion has been made to use this term specifically when the inhaled air contains more than 12 per cent of oxygen (WIGGERS 1941). This figure was chosen since observations were made which indicated that the types of circulatory adjustments varied above and below this point. No general agreement, however, has been met with in this respect (VAN LIERE).

As synonyms for anoxia, terms »oxygen lack», »oxygen deficiency» and »oxygen want» will be used. By analogy, term anoxemia will be used to designate the conditions of oxygen want in the blood.

Various types of anoxia

BARCROFT (1920) classified anoxic conditions into three main groups:

1. anoxic anoxia
2. anemic »
3. stagnant »

In addition to these, PETERS and VAN SLYKE (1932) have suggested and it has been generally accepted the recognition of a fourth type of anoxia, namely

4. histotoxic anoxia.

Anoxic anoxia

This paper deals solely with the anoxic type of anoxia. The main characteristics of this type of anoxia are a reduced arterial oxygen pressure and a low percentage saturation of the arterial and venous blood with oxygen.

The incomplete oxygenation of hemoglobin in the arterial system may be brought about by the following causes.

- 1). The inspired air is low in oxygen tension. This occurs at high

altitudes due to the fact that the partial pressure of oxygen gradually decreases with the increase of altitude. Breathing of gas mixtures low in oxygen percentage leads to the same condition.

2). The condition may be a consequence of inadequate oxygenation of the blood in the lungs even if the air contains normal oxygen values. Obstruction of the respiratory tract caused either by organic or functional changes or by the accumulation of foreign material in the passages results in lowered oxygen tension in the arterial blood. Processes in the lungs which reduce the functional respiratory surface there have the same effect.

It might be proper to point out in this connection that in certain conditions the severe obstruction of the respiratory passages can, in addition to the lowered arterial oxygenation, result in increased carbon dioxide contents of the blood and tissues. These conditions are specially referred to by the term asphyxia (GELLHORN and LAMBERT 1939, VAN LIERE 1942).

3). The arterial oxygen tension might be lowered also by congenital anomalies in the heart or by pulmonary shunt in which conditions venous blood is shifted directly into the arterial system without being oxygenated in the lungs.

The following discussion on the general effects of anoxia will be limited to treat experimental material accumulated under the conditions of anoxic anoxia. Furthermore, although no clear distinction can be made between the terms acute and chronic anoxia, only conditions in which no acclimatization has taken place will be considered. The discussion will thus be limited to anoxic conditions of short duration. Much of the earliest information has been obtained from observations made on mountain expeditions. Among the experimental means of producing anoxic anoxia, the use of low pressure chambers has proved to be a very valuable substitute for expensive and time consuming mountain journeys. The usage of rebreathers in which the expired air is conducted through carbon dioxide absorbents back to the air reservoirs and by which means the inhaled air becomes progressively deficient in oxygen has been used especially in animal experimentation. Finally, the breathing of prepared gas mixtures containing fixed amounts of oxygen presents a frequently employed means of producing anoxic anoxia.

Control of pancreatic secretion

Nervous control

The recent years have not added much to our knowledge of the nervous control of the pancreas. The observation made by the Pavlov school that

the pancreas receives both secretory and inhibitory fibres from the autonomic nervous system is generally accepted.

Parasympathetic control

Nearly all of the information regarding the parasympathetic control has been derived from animal experimentation. The apparent species differences which have been noted makes one cautious of applying the observations directly as such to human beings.

No disagreement exists on the question of the secretory effects of vagal stimulation in various species (PAVLOV 1888, ANREP 1916, BABKIN 1924, TONKICH 1924, BAXTER 1931, HEBB 1937, CRITTENDEN and IVY 1937, HARPER and VASS 1941).

On the other hand, the inhibition of pancreatic secretion caused by vagal stimulation which was described in dogs (PAVLOV 1888) and later confirmed by others (ANREP 1916, KOROVITSKY 1923, CRITTENDEN and IVY, 1937) can not be demonstrated in cats (HARPER and VASS 1941).

To the writer's knowledge, no work has been performed to establish whether the vagus contains inhibitory fibres in man.

Sympathetic control

The stimulation of the splanchnic nerves in dog may occasionally result in an augmented secretion and it may particularly increase the enzyme output by the pancreas (HEIDENHAIN 1875, KUDREVESKY 1890, SAWITSCH 1909).

The fact that under such conditions the effect can be abolished by atropine, but that it is not affected by ergotamine, together with the observation that the pancreatic veins were found to contain more acetylcholine after the splanchnic stimulation would indicate that the secretory fibres to the pancreas in the splanchnic nerves are cholinergic (THOMAS 1948).

On other occasions the stimulation of the sympathetic nerves results in an inhibition of the pancreatic secretion. This inhibitory effect of the sympathetic system on the pancreas has been demonstrated to be persistent in cats since the section of the splanchnic nerves results in a spontaneous secretion from the pancreas (HARPER and VASS 1941). The efferent pathways of this splanchnic reflex, are according to these investigators, obviously the splanchnic nerves.

Since vagotomy as well as the elimination of the possible inhibitory stimuli carried by the somatic nerves did not abolish the described effect,

the authors concluded that the afferent pathways for the inhibitory reflex also lie in the splanchnic nerves.

Humoral control

Secretin

The discovery of secretin as a link in the control of the pancreatic secretion as made by BAYLISS and STARLING (1902) has been generally recognized and accepted.

It has taken, however, several decades after this discovery firmly to establish the hormonal control of pancreatic secretion.

Later proof for the existence of a hormonal mechanism has been offered by crosscirculation experiments (MATSUO 1913), autotransplantation of the gland (FARRELL and IVY 1926, IVY, FARRELL and LUETH 1927, DELEZENNE, HALLION and GAYET 1927, HOUSSAY and MOLINELLI 1926) and by vividialysis (NECHELES and LIM 1928).

Also highly purified secretin preparations have been isolated (HAMMARSTEN, JORPES and ÅGREN 1932, GREENGARD and IVY 1938, GERSHBEIN and co-workers 1949).

The experiments of LANGSTROTH, McRAE and KOMAROW (1939) on the effect of secretin on the output and synthesis of protein material in the pancreas showed that both proceed independently of the parasymphthetic nervous system.

Doubt had also been cast by HAMMARSTEN, ÅGREN and LAGERLÖF (1937) upon MELLANBY's (1925) belief that secretin controlled only the fluid and alkali output of the pancreas whereas the vagus regulated the enzyme production. These observers by using purified secretin claimed to have shown in their work on man that secretin stimulates also the production of enzymes by the pancreas.

In some later work, LAGERLÖF and his co-workers found, however, that the secretion of the three chief enzymes after the administration of secretin was constant per unit of time, and therefore the conclusions were that the amount of enzymes secreted was independent of the secretin dose (LAGERLÖF and WELIN 1937, LAGERLÖF 1939 and 1942.)

On the other hand BARRINGTON (1941) studying the effect of secretin in cats maintained that secretin caused an augmented concentration and output of enzymes.

Pancreozymin

The opinion was held that the pancreas was under a dual control: the nervous system controlling the enzyme production of the gland, and the

humoral control being responsible for the water and bicarbonate production. This opinion was formulated by MELLANBY (1925) and has been held until recently.

Reports from various laboratories indicated that the question of the non-production of enzymes by secretin was not so clear. CHIRAY, JEANDEL and SALMON (1930) maintained that secretin after intravenous injection stimulated the enzyme flow and production in man. Similar observations were made by BARRINGTON (1941).

More crucial evidence against the pure nervous control of the enzyme production of the gland was offered by HARPER and VASS (1941). These investigators observed that in cats the passage of foodstuffs or water through the duodenum resulted in an increased enzyme output of the pancreas even after all the extrinsic nerves to the pancreas had been severed.

Such information at hand, added to the various reports of the effect of secretin prepared by different methods, stimulated HARPER and RAPER (1943) to find an explanation for the discrepancies.

This work led to the demonstration of a new hormone, which they called pancreozymin. The experiments which were performed on cats demonstrated that from the small intestine of the pig, dog and cat a substance could be extracted which acted on the pancreas to produce an increased enzyme output after intravenous injection. The volume of the juice was not effected by the preparation to the same extent. This substance therefore was not identical with secretin and was held responsible only for the enzyme production of the gland. A method for chemical isolation of such enzyme concentrates from the duodenal extracts was also described.

GREENGARD, GROSSMAN, WOOLLEY and IVY (1944) by studying the various fractions separated in the purification of secretin were able to confirm the findings of HARPER and RAPER to such effects that while other fractions contained very highly potent secretin material some other fractions were high in the enzyme producing agent.

In a study by using a constant secretin infusion in dogs WANG and associates (1948) showed that an increase of secretin administration causes an increase in the volume of juice but does not bring about an increase in the output of enzymes. Pancreozymin preparations, however, stimulated the enzyme output under these conditions as indicated by an increased concentration and total output of amylase.

In a more recent paper HARPER and MACKAY (1948) reported the effects of pancreozymin and of vagal nerve stimulation upon the histological appearance of the pancreas. Secretin brought about the secretion of a juice low in enzyme content and did not affect the granule content of the cells. The stimulation of the dorsal vagal trunk and the administration of pancreo-

zymin each resulted in an increased output of enzymes in the juice and a depletion in the zymogen granules of the acinous cells. The pancreozymin-produced effect differed from that evoked by the vagal stimulation, however, in the way that the former effect was not abolished by atropine.

In conclusion it may be stated that the hormonal control of the pancreatic activity is accomplished by two hormones, namely by secretin, which is responsible for the production of fluid and bicarbonate and by pancreozymin which is responsible for the production of pancreatic enzymes.

BABKIN (1950) has recently coined the terms »hydrelic» and »ecolic» to distinguish the two types of secretory functions of the pancreas. Using these terms it can be said that secretin stimulates the hydrelic function whereas pancreozymin stimulates the ecolic function of the gland.

Previous studies on the effect of anoxic anoxia on the digestive glands

Salivary secretion

It has been shown that the activity of the salivary glands is associated with an increased oxygen consumption and carbon dioxide production (CHAUVEAU and KAUFMAN 1886, ANREP and CANNAN 1922, 1923). These facts with the observation that the salivary secretion is associated with an increased glucose consumption of the glands (ANREP and CANNAN 1923) indicate that the secretory process of these glands is not simply a filtration from the blood stream.

Studies on the sensitivity of these glands against inadequate oxygen supply have been made. The mixed type of glands, namely the submaxillaries have been the most commonly used object for the observations.

Working on anesthetized dogs and stimulating the submaxillary gland with constant administration of pilocarpine (in 1:100000 solution) EDDY (1929) maintained that the reduction of the O_2 contents in the inspired air to 18 per cent resulted already in a decreased rate of secretion. If the administration of a very low oxygen content (7 per cent) was prolonged the rate of secretion, however, began to increase. These changes were independent of the changes in the blood pressure. In his experiments an interesting phenomenon was also noted, namely that in one experiment when the systemic blood pressure during low oxygen inhalation suddenly fell abruptly there occurred a secondary increase in the otherwise low rate of salivary secretion. The observation period in these experiments was relatively short, the exposure to oxygen lack being only 5 minutes.

BRODY and SHILING (1930) also working on dogs and observing the submaxillary secretion, maintained that the type of stimulus applied in producing the secretion was of importance. They confirmed EDDY's work showing that if pilocarpine was used the breathing of 10 per cent oxygen mixture reduced the secretion to 50 per cent or less, while the response to an electrical stimulus applied through the chorda tympani showed no change under the anoxia conditions.

A reduced response during oxygen lack by the salivary glands has also been reported by BRASSFIELD (1936), KRESTOVNIKOV and MAZNICHENKO (1938) and FILIPPOVICH (1940).

The two latter groups made observations also on man. KRESTOVNIKOV and co-operators reported reduced responses to 1 per cent citric acid stimulation at an altitude of 3630—4000 meters as compared to the responses produced at sea level.

FILIPPOVICH reports after effects after 4—5 hours exposure to oxygen lack, this after effect lasting in man from 4 to 7 days and making its appearance also in form of dryness of the mouth.

STREL'COV (1943) reviewing the work done in the Soviet aviation laboratories states that the response of the salivary secretion to lowered atmospheric pressure takes appearance in two phases. First, under slight degrees of anoxia there might be some increase in the secretion. Later, and with more severe anoxia, there is a great suppression. The conclusions were drawn that the reduced salivary secretion is caused by disturbance of the nervous regulation of the glands.

Gastric secretion

Several studies have been made on the gastric secretory functions during anoxia.

The first of such observations were made on dogs in which BAYEUX (1911) showed that the quantity of juice secreted after a given test meal at a simulated altitude corresponding to the height of 4300 meters was considerably less than when the same experiment was done at sea level. The total output of acid was, however, found to be only slightly affected according to this report.

These findings, particularly those regarding the volume output, have been repeatedly confirmed both by animal experimentation (DELRUE 1934, SLEETH and VAN LIERE 1936, PICKETT and VAN LIERE 1939, SELMANOVA 1940) and in human beings (WARREN 1937, HARTMAN, HEPP and LUFT 1941, HARTIALA and KARVONEN 1946, KARVINEN and KARVONEN 1949). It has been demonstrated by these studies that the HCl-secretion is

suppressed by anoxia. The only contradictory results in this connection have been reported by HELLEBRANDT, BROGDON and HOOPES (1935). These investigators who used a rebreathing method and severe degrees of anoxia were still unable to detect any changes in the HCl-secretion by the stomach.

Claims that the gastric secretory suppression induced by anoxia might be due to the concomitant alkalosis under the experimental conditions (WARREN 1937, HARTMAN, HEPP and LUFT 1941) have been contradicted by the studies of HARTIALA and KARVONEN (1946). These authors were able to show that the prevention of alkalosis by administration of acidifying salts — NH_4Cl — prior to the anoxic exposure could not prevent the anoxic suppression of the HCl-secretion.

Reports on studies using innervated and enervated gastric pouches on dogs have shown some contradictory results as regards to the behaviour of the two types of preparations during anoxia.

Although in both types the volume of gastric juice decreased from that of the control levels, the Heidenhain type of pouch (denervated) was affected more by anoxia than was the PAVLOV (innervated) pouchgroup according to PICKETT and VAN LIERE (1939).

SELMANOVA (1940), although confirming the decrease in both types, found in contrast to the former investigators that the innervated stomach was more sensitive to anoxia than the denervated one. It is of interest to notice that in the latter experiments with the innervated pouches both the cephalic and gastric phases of the secretion were equally affected by the tested altitude of 6000—8000 meters.

As to the other components of the gastric secretion only very few studies have been made.

The pepsin output has been studied by GIANOTTI and GOLDBERGER (1931) and more recently by KARVINEN and KARVONEN (1949).

The interpretation of the data reported by the former group is complicated by the experimental procedure employed, the subjects having performed strenuous exercise during these experiments.

The latter investigators, however, were able to detect a considerable reduction in the pepsin output in resting human subjects as indicated by the response to a vagomimetic or alcohol stimulation at sea level and at a simulated altitude of 5000 meters.

Intestinal secretion

From the very few studies made on this subject it would appear that the intestine as a secretory organ is rather resistant to low oxygen environment.

NORTHUP and VAN LIERE (1939) working on anesthetized dogs and

studying the jejunal response to a peptone stimulation were unable to detect any significant changes in the secretion at a simulated altitude of 5400 m, and only at a very extreme altitude (8400 m.) was the secretion suppressed.

KHASEN (1940) reports work done on dogs with Thiry-fistulas and denervated intestinal fistulas. During a 6 hours exposure to a simulated height of 6000 m. there was an initial decrease of secretion in both types of preparations which effect later was substituted by an increase. The enzyme concentrations in the intestinal contents under these conditions showed an increase, probably due to the smaller amount of fluid present. There were also increased urea contents in the juice. The enervated preparation did not show the initial decrease.

VAN LIERE (1942) comments that the experiments so far performed seem to indicate that the intestine has a low energy requirement and is not readily affected by oxygen lack.

Bile secretion

Only few studies have been performed dealing with the bile secretion and formation.

TANTURI and IVY (1938) studying the bile secretion in bile fistula dogs report that they were unable to find any definite changes in the bile flow during the 20 minute observation periods. The anoxic effect was produced by breathing air containing 7.5—10 per cent oxygen.

Similar conclusions were drawn also by MACLACHLAN et al. (1947) who made the observations on rats.

On the contrary to these two reports GLIKSON and co-workers (1940) who made their observations on a bile fistula dog at a simulated altitude of 6000 and 8000 meters report a decrease in the bile flow as compared to the control secretion on sea level conditions. The decrease affected mainly the amount of fluid while there was an increase in the viscosity of the juice and bile acid and pigment contents in it. The cholesterol contents were reduced.

SCHNEDORF and ORR (1941) also working on dogs report a marked decrease in the bile flow during anoxia although there did not appear to be any changes in the bile salt excretion.

Pancreatic secretion

Very little attention has been paid to the secretory functions of the pancreas during anoxic conditions. The only investigation, to the author's

knowledge, on this function has been made by RASENKOV. The observations were made during a mountain expedition. According to the report the pancreas under those conditions secreted more bicarbonate than usual. This phenomenon in addition to the reduced acid liberation by the gastric glands has been interpreted as an additional compensatory measure of the organism against the change of the acid — base balance (STREL'COV 1941).

Unfortunately, the original paper has not been available to the writer. The short abstract on the described observations does not give any numerical data. Furthermore, no information is available on how the experiments were performed, what stimulus was employed etc.

Apart from this paper no other reports on the subject have been available, and to the author's knowledge no other reported observations on the subject have so far been made.

Plan of the study

According to the described report on the pancreatic secretion (RASENKOV) it would appear that this gland is not readily affected by anoxia but, by virtue of its alkali constituents, would serve as a compensatory organ to maintain normal acid-base conditions in the body.

In this respect it would differ greatly from the salivary and gastric secretory functions which were shown to be suppressed by oxygen lack, irrespective of concomitant changes in acid base balance.

The present studies were undertaken in order to elucidate these facts by applying the modern techniques for studying the pancreatic function in man.

The pancreas is under both nervous and humoral control. This research project was limited, however, to the study of the effects of anoxic upon the gland as stimulated humorally.

The following problems were presented.

1. Does the pancreatic secretion constitute a compensatory mechanism in the maintenance of the acid-base balance, even at severe degrees of anoxia?

2. If the alkali secretion is found to be increased in relation to the compensation functions the gland might have, what is the response of the enzyme production under the same condition?

3. If the pancreatic secretion is similarly affected by anoxia as are the secretions of the salivary and gastric glands, does the human pancreas in alkalosis produce more alkali than usual?

During the course of the work it was found that, owing to the nature of the employed pancreatic function test, a detailed view of the secretory pattern of the pancreas could not be obtained.

In order to fulfil this requirement a series of experiments on dogs was undertaken.

Since the experimental procedures now could be so designed as to give more closely continuous records on the secretory rate of the various constituents of the pancreatic juice, additional questions were presented.

4. Can the same effects of anoxia on the pancreatic secretion as was found in the human experiments be demonstrated in anesthetized dogs applying a constant hormonal stimulus?

5. What is the detailed pattern of the alkali and enzyme secretion during and after the anoxic exposure?

Chapter II Human experiments

Methods

Historical

By analysing the indirect methods proposed for the studies of the pancreatic secretion it would appear that none of them would be suitable for quantitative studies on the activity of the gland in experiments of short duration. The most serious handicap of these methods as regards their usefulness for the planned study, however, is that with them only organic changes in the organ or obstruction of the secretory passages can be measured.

It was therefore found desirable to employ a more sensitive direct method for the purpose.

By the direct methods the pancreatic juice itself is subjected to analyses.

Collections from an open pancreatic fistula yields uncontaminated material which can be analysed. At least one such study has also been made in our country (HOLSTI 1913).

On all other occasions the juice is contaminated by other duodenal secretions and bile, and in the older type of work also by gastric contents.

The first observations with regard to the external pancreatic secretion in man, were based on analyses of the regurgitated duodenal contents collected from the stomach (BOAS 1889, ELLENBERGER and HOFMEISTER 1891).

A further step was the introduction of a tube into the duodenum which was first attempted by HEMMETER (1896) and later successfully by improved devices achieved by EINHORN (1910).

A further advance was the use of two tubes, the longer one being

introduced into the duodenum, and the other remaining in the stomach. Such experiments were first reported by BÄRSONY and EGAN (1922) who used tubes which were tied to each other by means of metal rings. CARNOT et al. (1922) employed two separate tubes. They were not, however, always successful in separating the gastric contents from the duodenal contents.

LIM and co-workers (1923) employed a new double tube method (single tube with two lumens). They also emphasized the necessity of a constant suction through the tubes in order to prevent regurgitation of duodenal contents into the stomach and vice versa.

Despite the obvious inaccuracy which necessarily accompanies every procedure employing a single tube with one lumen, such techniques are still occasionally in use. Such experiments would serve merely as an indication of a functioning pancreas without giving base for quantitative analysis. Not only do experiments performed by this way deal with salivary and gastric secretions but they also involve the varying degrees of stimulatory effects of these agents on the release of secretin and pancreaticozym and thus on the pancreatic secretion in addition to the original stimulation.

The question of an adequate stimulus for the pancreas can now also be considered established.

In the earlier work a great variety of stimuli were recommended. Various foodstuffs have been employed for the purpose, e.g. peptones (STEPP and DÜTTMAN 1923, DELOCH 1922). Tea has also been used (BONDI 1923).

Chemical agents such as $MgSO_4$ have been commonly used (DELOCH 1922, MAGNANO 1935). Aether has also been known to evoke a stimulation of the pancreas (KATSCH and FRIEDRICH 1922).

The credit for using secretin first in man belongs to CHIRAY, MERCIER and SALMON (1926). These investigators collected the juice secreted after secretin stimulation over 5 minute periods during 25 minutes. They also attempted to evaluate the analyses for diagnostic purpose.

The experiments to be reported in this paper are made according to the technique described by LAGERLÖF (1942). This method will now be discussed in detail.

Collection of the pancreatic juice

The drainage of the duodenal contents was achieved by means of a double tube technique. For this purpose two separate tubes were vulcanized together in the following manner.

One tube, being a regular size duodenal tube, was fixed together with a

shorter stomach tube so that the lower terminal end of the thinner tube exceeded by exactly 30 cm. the length of the shorter one. The outer diameters of the tubes were 5 1/2 mm. and 6 1/2 mm. The inner diameter of the duodenal tube was 3 mm.

The duodenal portion of the tube was perforated with small holes. After few experimental trials it was found that when using large holes in the tube it became easily obstructed by mucus. This difficulty could be avoided if the perforations were made small so that such holes were made which were less than the diameter of lumen in the tube. The holes were perforated along the last 20 cm. of the terminal portion of the tube. Thus when properly placed in the duodenum the pylorus region of the tube was not supplied by perforations in the tube.

In the stomach portion of the tube the perforations were made accordingly to the described dimensions.

In order to achieve a rapid passage of the pylorus in the stomach a metal ball attached to the duodenal tube has been recommended by KÜNSZTLER (1930). Such device belongs also to the procedure employed by LAGERLÖF.

In this work a gold-plated copper ball was used for the purpose. The diameter of the ball was 7 mm. The weight was attached to the duodenal tube by means of a silk thread.

From the first experimental trials it appeared that the silk thread connection between the gold plated ball and the duodenal portion of the tube lead to injury of the mucosa since the samples collected through the system were not infrequently contaminated by blood. The back and forth movements of the tube could cause the sharp edge of the silk thread to cut the mucosa.

This harmless though disturbing phenomenon could to the writer's experience be eliminated by covering the silk thread with a thin plastic or rubber tubing. After using this extra rubber tubing the duodenal and gastric contents did not contain blood.

Another and more important modification made its use essential due to the experimental conditions to be used. The use of a constant suction by means of either a water suction pump or any other mechanical device surely must be appreciated. However, no such device could be attached to the only type of low pressure chamber available. Since efforts were made to keep the experimental conditions during the various phases of the work as similar as possible, the suction of the gastric and duodenal contents was achieved by large syringes of 50 ml. capacity, attached to the tubes. A continuous negative pressure was kept in the syringes so that practically continuous suction was maintained in the system. The pressure in the system was not, however, kept constant by this means. Whether this fact

bore any significance on the results will not be established here. The possibility of this being one of the factors resulting in a slightly lower average response to the secretin as compared to the results reported in LAGERLÖF's material will be considered in the discussion of the results.

Stimulus applied

The secretin preparation »Pancreotest» manufactured by the Astra Co in Södertälje, Sweden, was used throughout the work.

According to ÅGREN (1934) and LAGERLÖF (1942) this preparation undergoes a gradual deterioration in time resulting in a lose of its potency. The loss of activity is constant per unit of time. The possible effect of the inactivation on the yield of pancreatic juice obtained using this preparation will be discussed later.

A constant dose of secretin was given to each subject. This dose was calculated per kilos of body weight so that each subject received 1 clinical unit of secretin per kilo.

Before administration, the secretin was dissolved in normal saline solution to obtain a concentration of ten units per cubic centimeter. The solution was heated twice to boiling accordingly to the instructions given by the manufacturer. After being cooled to room temperature it was slowly injected into the cubital vein of the subject.

Procedure of the secretin test

The ball attached to the tube was first swallowed by the subject. The tubes were than introduced into the stomach up to the mark of 70 cm. measured from the lower end of the duodenal tube.

After this the subject was placed on his right side and it was expected that the duodenal tube could be pushed forward without feeling any resistance on account of the closure of the pyloric sphincter. The passing of the gold-plated ball as well as the duodenal portion of the tube was in general achieved without difficulties. The range for the passage varied from 15 minutes to three hours. On a few occasions, however, the procedure did not succeed on account of cardiospasm which made it necessary to interrupt the attempts for that day.

The longer tube was, after have passed the pylorus, pushed down to the 90 cm. mark on it. The final position of the tubes in the stomach and duodenum were now such that the terminal end laid in the region of the duodeno-jejunal flexure, the unperforated area of the same tube in the

pyloric region. The shorter tube was in the stomach, the tip of it being in the pyloric region.

When at its proper location, the aspiration of the duodenal tube should yield duodenal contents which are characterized by the color given to them by the bile pigments. On the other hand when the suction is maintained, the gastric contents aspirated from the shorter tube should not contain duodenal contents and would therefore be colorless.

Since there were no X-ray facilities in the laboratory the correct location of the tubes was assured solely on the nature of juice aspirated through them. No test was performed before it was certain that the duodenum did not contain gastric contents and vice versa. Fluoroscopic examination of the situation would in many instances have been desirable in order to save time and trouble. In case of doubt it was preferred to save the secretin and discontinue the experiment for the day.

Although no analyses were made on the basal secretion obtained prior to the secretin stimulation, care was taken to be sure that this did not exceed the rate of 1 cc. per minute.

After the secretin injection the juice was collected during a period of 60 minutes. The juice was divided into 4 portions corresponding to the yield obtained during the two first ten minute periods and the two following twenty minute periods. Each fraction collected this way were kept separately and subjected to further analyses.

Analyses of the pancreatic juice

The juice was collected into small flasks and immediately diluted with equal volume of glycerin. This measure was taken to protect the enzymes and especially trypsin from inactivation (LAGERLÖF 1942). The samples were then immediately placed into a refrigerator. In the case of the low pressure chamber experiments, the cooling effect was obtained by first placing the samples into a metal carrier containing ice, and then, after return to the laboratory placing them into the refrigerator. The pancreas supplies enzymes for the digestion of the three kinds of foodstuffs, carbohydrates, proteins and fats. It would have been ideal to study all of these enzyme systems. Unfortunately, however, only two of them could be analysed in this work, namely amylase and trypsin. As to the normal control material, this does not bear any great importance since the parallel secretion of the three enzyme systems has been established (SAVITCH 1909, ANREP, LUSH and PALMER 1925, BAXTER 1931 and 1935, HARPER and VASS 1941 and LAGERLÖF 1942).

It is only in the pathological conditions that a dissociation of the

production of the enzymes has been demonstrated (LAGERLÖF 1942). Such dissociation could possibly occur under the conditions of anoxia.

The lack of suitable reagents at the time the work was planned did not allow the analyses of lipase. It is obvious on the other hand that the pancreatic lipase under the conditions in which it is recovered from the duodenal contents is very rapidly inactivated. (WEINSTEIN and WYNNE 1935, BAMANN and LAEVERENZ 1934, LAGERLÖF 1942).

According to the latter investigator the inactivation of the lipase before the addition of glycerin was about 20 per cent in 5 minutes and about 50 per cent in 15 minutes. It becomes evident then that a great loss of activity takes place before the samples can be placed into glycerin and cold. Since the rate of secretion differs greatly during the various phases of the work it would be rather difficult to obtain reliable information on the real quantities of lipase activity produced by the gland under these conditions. Also the pH of the juice has its influence on the rate of inactivation according to the referred reports. The pH of the juice, on the other hand, increases when more bicarbonate is secreted, in other words, when the hydrelatic function of the gland is increased. All these factors added together discouraged the writer from performing the lipase determinations with the facilities available during the experimentation.

The bicarbonate content of the juice as well the amylase and trypsin contents of it were subjected to further analyses.

B i c a r b o n a t e

The bicarbonate contents of the juice were estimated by indirect titration. After adding a constant amount of 0.1 n HCl to it, the mixture was heated to evaporate the liberated carbon dioxide produced by the following reaction.



The excess acid was then titrated with 0.1 n NaOH. Phenolphthalein was used as an indicator.

A m y l a s e

The NÖRBY (1935) method as it has been modified by LAGERLÖF (1942) was employed for the amylase determinations. This method applies soluble starch as the substrate. The amount of maltose hydrolysed by the amylolytic enzymes is determined according to the well-known HAGEDORN-JENSEN method (1923) for blood sugar determinations.

P r o c e d u r e

The duodenal juice is diluted with a 1.5 per cent sodium chloride solution. The chloride was added on account of the activation of this agent on the amylase (BIERRY and GIAJA 1906, WILLSTÄTTER, WALDSCHMIDT-LEITZ and HESSE 1923). In order to avoid the error of using too active enzyme concentrations, different dilutions were used for the samples collected during the two first ten minute periods after the secretin stimulation. These portions, as will be seen later, are high in enzyme contents. The following dilution scheme was used therefore throughout the whole experimentation in order to have the corresponding portions in all phases of the work analysed the same way:

sample 1, (10^1)	1:800
» 2, (10^2)	1:800
» 3, (20^1)	1:400
» 4, (20^2)	1:400.

2 cubic centimeters of the dilution were placed into a test tube and kept in a water bath at 37 degrees C for eight minutes. The prepared starch solution containing 5 gm. of soluble starch (KAHLBAUM), dissolved in equal volumes of distilled water and 1/15 molar phosphate buffer ($\text{Na}_2\text{HPO}_4 \cdot 2 \text{H}_2\text{O}/\text{KH}_2\text{PO}_4$) was simultaneously heated to the same temperature. 2 cc. of the solution was added to the enzyme mixture and the tube was shaken by hand several times to assure a homogenous mixture. Exactly after 15 minutes, the time being noted by a stop watch, 2 cc. of the mixture was transferred into a Hagedorn tube containing 1 cc. of tenth normal sodium hydroxide. The hydrolysis was now ended.

The liberated maltose was then analysed by adding 2 cc. of 1/20 normal alkaline potassium ferricyanide and 12 cc. of distilled water and the tubes were placed in a test tube rack in a boiling waterbath. After exactly 20 minutes, they were taken out and cooled immediately by placing them in another cold water container. After the samples were cooled, 2 cc. of 50 per cent acetic acid and 10 cc. of a solution containing 25 mg. of potassium iodide, 250 gm. of sodium chloride and 50 mg. of zinc sulphate per liter was added. The excess potassium ferricyanide which had not been reduced by the maltose reacted with the potassium iodide and the liberated iodine produced by this reaction was titrated with 1/20 n. sodium thiosulphate.

A blank test was performed by adding 1 cc. of duodenal juice dilution and 1 cc. of the starch solution directly into the Hagedorn tubes containing 1 cc. of the sodium hydroxide solution. The procedure was from this point on the same as just described.

The amount of the reduced potassium ferricyanide could now be determined by the difference between titration values for the blank and the active sample.

The amount of maltose produced by the process can be calculated when knowing that 1 cc. of the used ferricyanide corresponds to 2.15 mg. maltose.

The *units* chosen for expressing the amylolytic activity of the samples were also the same as used in the original work of LAGERLÖF. Thus one unit of amylase activity is defined as the amount of amylase of which one thousandth gives the reaction constant of 0.001 during the described conditions.

It has been shown by previous workers (WILLSTÄTTER, WALDSCHMIDT-LEITZ and HESSE 1923) that the splitting of starch with pancreatic amylase follows a monomolecular course and that the reaction constant is proportionate to the amount of enzyme employed. It can therefore be used as an expression for the amylolytic power. Multiplication of the reaction constant with the dilution gives the number of amylase units per cubic centimeter. For the conditions of the present study the reaction constant can be calculated from the following formula:

$$C = \frac{1}{t} \log \cdot \frac{7.5}{7.5 - a},$$

where t stands for time (15 minutes). 7.5 is the amount of maltose formed maximally from the 10 mg. of starch (v. EULER and SVANBERG 1920—21), and a stands for the amount of maltose actually formed during the hydrolysis.

The calculation of the units was made by using graphs in which the titration values of $\text{Na}_2\text{S}_2\text{O}_3$ directly gave the amount of amylase units per cubic centimeter of duodenal juice. Such graphs were calculated separately for the two different dilutions of the juice used in the work.

Trypsin

The trypsin such as it appears in the duodenal content needs no further activation, being already activated by the enterokinase produced by the duodenal glands. According to CHRISTANSEN (1933) the activation of the pancreatic trypsinogen in the duodenum by enterokinase is complete and cannot be potentiated by any additional measures.

The original principle for determining the tryptic activity such as it has been described by WILLSTÄTTER, WALDSCHMIDT-LEITZ, DUÑAITURRIA and KÜNSTNER (1926) was employed. It is based on the titrimetric estimation of the tryptic digestion. In this particular work the analyses were done by using a 6 per cent casein solution buffered to a pH of 9 and carrying the

hydrolysis at a 30 degree C temperature. The basic properties of the amino-acid groups can be suppressed by adding heated alcohol up to 90 per cent of the mixture.

Procedure

2 cc. of the duodenal sample were heated in a water bath to 30 °C. Exactly five minutes and thirty seconds later 2 cc. of a buffer solution was added. (The buffer was made from 120 cc. each of normal ammonium chloride and ammonium hydroxide plus 160 cc. of distilled water). Exactly 30 seconds later 3 cc. of casein solution containing 30 mg. of casein mixed first with 300 cc. of water, with the addition of 20 cc. of normal ammonium hydroxide solution and after shaking made up to 500 cc., was added to the enzyme buffer solution.

The tubes were shaken vigorously after the addition of the casein solution. Exactly 20 minutes later the contents of the tubes were removed and placed in 250 cc. Erlenmeyer flasks containing 38 cc. of 95 per cent alcohol, 3 cc. of water and 1 cc. of 0.5 per cent alcoholic thymolphthalein indicator solution.

The contents were now titrated with 0.1 n potassium hydroxide solution until the appearance of blue color. The basic aminogroups were suppressed by adding 72 cc. of boiling 95 per cent alcohol mixture. The color disappeared and the titration was again continued to the same blue color.

A blank was set up containing the same amounts of the reagents in the Erlenmeyer flask. To this exactly the same amounts of duodenal juice, buffer and casein solution were added and the titration was performed as described above.

The difference between the titration values for the digested sample and the blank gave the amount of carboxyl groups liberated by the enzymes in the sample.

One *trypsin unit* under these conditions, according to LAGERLÖF, corresponds to the titration value of 1.73 cc. of 0.1 n KOH solution.

The unitage of the analysed samples was obtained from a graphic curve in which the cubic centimeters of tenth normal potassium hydroxide were placed on the ordinate and the trypsin units on the abscissa. The unknown units could now be easily determined from the titration values.

Comment on the trypsin method

In contrast to the amylase determinations, the trypsin analyses performed according to the described method leave much room for improvements. One of the main difficulties in this method is the exact determination of the endpoint for the titration. Since

the duodenal samples contained varying amounts of bile, this factor constituted a difference in the original color appearance of the samples. In the presence of the bile pigments no blue colour could be obtained during the titration, the endpoint under such conditions being light green. The titration of such samples were apt to make the titration a rather unpleasant experience. This complication calls, to the writer's understanding, for the use of some other method and preferably for the use of a method in which the end results could be analysed more specifically.

Method for producing anoxia

The anoxic effect was produced by using a low pressure chamber. The dimensions of the chamber were such that it accommodated well four persons at once. No more than two persons, however, were in it during each experiment, the test subject and the conductor of the experiments.

The control of the pressure in the chamber was done by a technician outside the chamber. The existing pressure could be simultaneously read both inside and outside the chamber. The pressure was kept at the decided level within a range of 10 — 15 mm. Hg.

Two different degrees of anoxia were applied. One of them corresponding to an altitude of 5500 m. and a barometric pressure of 382 mm. Hg, and an oxygen partial pressure of 80 mm. Hg, was taken to present the severe degree of anoxia.

The other level was fixed to correspond to the altitude of 3500 and 4000 meters. The barometric pressures at this level correspond to 480 and 450 mm. Hg pressure and to a partial oxygen pressure of 101 and 94 mm. Hg respectively.

Especially at the higher levels of simulated altitude the visual characteristic features of anoxemia manifested themselves. The degree of cyanosis seemed to vary in different subjects. No special consideration to these observed changes in the color or respiration of the subject was given as long the subject did not claim any alarming discomfort. Fortunately enough, all the subjects tolerated the anoxic exposure without complications so that the disposed oxygen masks were not needed. In order to distract the subject from his condition some interesting reading material was always taken with him into the chamber. The subject was seated on a chair as in the control experiments.

Material and experimental procedures

Test subjects

The experiments were performed on 14 young healthy medical students, thirteen male and one female. The age of the subjects ranged between 19

and 25. The subjects were chosen on the basis of their willingness voluntarily to co-operate in the experiments. Care was taken that none of the chosen subjects had a complicating respiratory, circulatory or gastrointestinal disorder in their history, and that at the time of the experimentation they appeared to be in good health.

Experiments

Two possibilities were at hand when planning the experiments. Each subject could be tested twice for control observations and again twice under anoxic conditions. The other possibility, namely a single control and anoxic experiment, was chosen mainly for the following reason.

Only a small amount of secretin was available for the study. The shortage of secretin therefore limited the number of objects to be tested. According to the records available from the students of the subject reliable evidence, however, has accumulated to the effect that the response of the same person to repeated injections of similar doses of secretin remains practically the same (ÅGREN and LAGERLÖF 1936, HAMMARSTEN, ÅGREN and LAGERLÖF 1937, LAGERLÖF and WELIN 1937, LAGERLÖF 1942).

It was therefore reasoned that any significant changes caused by anoxia could be detected by this way even after a single test.

According to this reasoning the advantage could be achieved of investing the limited amount of secretin in a larger group of individuals.

The *control experiment* on each subject was always performed before the anoxic. This was done at sea level conditions. If the procedure succeeded the anoxic experiment was then done a few days after this.

The tests were always performed after a fasting period of 15 hours and in the morning.

The *anoxia experiments* were performed in the following way. The tube was introduced into the proper location before entering the low pressure chamber. After being seated in the chamber the ascent to the desired simulated altitude was done. This was only a matter of minutes. The secretin was not, however, administered immediately, in order to allow the subject to become used to the condition. After now being acquainted with the procedure and after having secured a low basal secretion from the pancreas, the secretin was injected and the procedure continued exactly as in the control experiments.

It should be emphasized that the test subject was exposed to the anoxia before the secretin was administered and that the whole function test, therefore, was performed under the same degree of anoxia.

The length of anoxic exposure together with the ascent and descent was

approximately 100 minutes in each case. The secretin was injected between the first 20 — 30 minutes after the beginning of the anoxic exposure.

Blood sugar

Samples for the few blood sugar determinations were taken from the finger tips and analysed according to the method of HAGEDORN-JENSEN (1923). Such samples were taken immediately before entering the low pressure chamber and immediately after the secretin test either in the chamber already or few minutes after leaving it.

Method for producing alkalosis

One of the most commonly used means of producing experimentally alkalotic conditions is to ingest alkalinising salts. It has been shown that the oral administration of 5—10 gr. of sodium bicarbonate is sufficient to make the urine strongly alkaline in a normal individual (SELLARDS 1912, PALMER and HENDERSON 1913). The maximal alkalinity is reached in about 1 hour and the effect lasts for few hours.

According to HALDANE (1924) a daily dose of 45 grams of the same drug or equivalent amounts of some other alkalinizing salts (e.g. sodium acetate) is required to maintain the urine continuously alkaline and maintain an excess bicarbonate level in the blood. As a result of such treatment the blood carbon dioxide capacity increases, the alveolar carbon dioxide pressure increases, there is a rapid occurrence of bicarbonate in the urine and a reduction of ammonium secretion by the kidneys (DAVIES, HALDANE and KENNAWAY 1920).

The following treatment was used in these experiments. The subjects took sodium bicarbonate tablets the night before the pancreatic function test was performed and in the morning of the same day according to the scheme:

- 6 gm. before retiring in the night,
- 10 gm. between 6.00 and 6.30,
- 10 gm. between 7.00 and 7.30 and
- 10 gm. between 8.00 and 8.30 in the morning.

Tablets containing 0.5 grams of sodium bicarbonate were used. Since the simultaneous ingestion of food has been found to be favourable in preventing the purgative action of the large amounts of the salt used, the first tablets were taken after a light meal. The ingestion of food was not

however desirable immediately before the duodenal drainage, and since the 15 hrs fasting period was still maintained, the purgative side-effect of using the salt could not be avoided. The last tablets were taken in the laboratory after the subject had arrived there. The double tube was inserted in its right position after one hour's rest in bed. All the drug still present in the duodenum and stomach was removed and the tubes rinsed with water before the collection of the basal secretion was begun.

The pancreatic function test performed now was in other respects the same as in the other parts of work.

Determination of the effects of the administered bicarbonate

Alveolar air

The alveolar air samples when properly taken are shown by comparison with direct analyses of the blood to have partial carbon dioxide pressures which correspond to those in the arterial blood (KROGH and KROGH 1910, CAMPBELL and POULTON 1920, PETERS, BARR and RULE 1921, BOCK and FIELD 1924, HENDERSON, BOCK, FIELD and STODDARD 1924, DILL, HURXTHAL, VAN CAULAERT, FÖLLING and BOCK 1927, DILL, LAWRENCE, HURXTHAL and BOCK 1927, COMROE and DRIPPS 1944). Analyses of the alveolar air for its carbon dioxide contents serve therefore as an accurate enough substitute for the direct analyses of the arterial blood. The choice of using such method was done in order to receive information on the effect of the drug on the subjects at several intervals, and yet disturbing the condition of the subject as little as possible. Although a simple procedure, arterial punctures, when performed several times on the same person within short intervals, was not considered desirable.

The principle of HALDANE and PRIESTLEY (1905) was employed for the purpose. This procedure requires rather intelligent co-operation on the part of the test object in order to maintain respiration unchange until the collection of samples. The medical students serving as the test material learned the procedure after a brief training period during which emphasis was laid on keeping relaxed, and despite the manipulations to maintain the usual respiratory pattern and follow exactly the orders of the conductor of the experiments while collecting the samples.

The method employs a metal tube approximately 100 cm. long provided with a mouthpiece and a short side tube near the mouthpiece. The side tube was connected to gas analyses collection flasks which had been evacuated of air. The samples were taken after a normal expiration had been completed by a forced expiration.

There are two ways of collecting the samples and determining the alveolar air contents: a) by collecting the so-called inspiratory alveolar air. This is obtained at the end of a forced expiration after a normal inspiration; b) by collecting the expiratory alveolar air which is obtained at the end of a forced expiration started at the end of a normal expiration. The latter procedure was used in the present work.

According to comparative analyses made by using either one of these methods it has been established that in resting subjects the expiratory alveolar air agrees better with the arterial blood in respect to its carbon dioxide contents than do the two other possibilities, namely, the inspiratory alveolar air or the average of the two samples (MEAKINS and DAVIES 1920, PETERS, BARR and RULE 1921, DILL, HURXTHAL, VAN CAULAERT, FÖLLING and BOCK 1927).

Since the experiments in the present work were performed on resting subjects the expiratory type of collection was chosen.

The gas samples were analysed by the HALDANE gas analysis apparatus to their carbon dioxide contents.

U r i n a r y p H

The urine was collected into a 1000 cc. flask containing 10 cc. of liquid paraffin to prevent the loss of carbon dioxide from the samples. 1 cc. of toluol was also added into the bottles to preserve the urine from bacterial decomposition.

The pH determinations were made by means of a glass electrode and a valve potentiometer.

Statistical methods

The following abbreviations are used in the formulas expressing the mathematical treatment given to the results:

n = number of observations

x = a single observation

$\bar{x}$ = the arithmetical mean of the observations

b = difference between two observations

St. D = standard deviation

St. E = standard error of the mean

The formulas used for the calculations were:

$$\text{St. D} = \sqrt{\frac{\sum (x - \bar{x})^2}{n - 1}}$$

$$\text{St. E} = \frac{\text{St. D}}{\sqrt{n}}$$

The difference between the means of two series observations was analysed according to the t-test described by »Student». The formula thus employed was:

$$t = \frac{(\bar{x}_1 - \bar{x}_2)}{s} \sqrt{\frac{(n_1 + 1)(n_2 + 1)}{n_1 + n_2 + 1}}$$

where s is calculated from the equation:

$$\sqrt{\frac{1}{n_1 n_2} \{ \sum (x_1 - \bar{x}_1)^2 + \sum (x_2 - \bar{x}_2)^2 \}}$$

In this equation n_1 and n_2 stand for the individual observations in two series minus one.

Results

Control experiments

The secretin test was attempted on 14 subjects. One of these, the writer, was subjected to it several times in order to learn the procedure as well as to establish the reliance of the test. During this practice different kinds of double tubes were tested. Since the original tube, the holes in which were large and irregularly scattered, seemed easily to cause an obstruction of passage it was changed to the type of tube described in the methods. The yield with this tube was found to be of approximately the same magnitude as reported by LAGERLÖF. The same tube was then used throughout the whole experimentation.

A successful test both at atmospheric as well as at simulated altitude was achieved in 10 of the subjects. From the remaining three subjects, the anoxia experiments were not performed on them, nor on the writer, as one collapsed during the first control test (perhaps due to a too rapid administration of the secretin) and it was thought to be a risk to allow him

into the low pressure chamber, and no further tests were performed on him. For the purpose of curiosity it might be mentioned that the yield of this collapsed subject was extremely low.

In the other two subjects, either one of the tests was not a satisfactory one, due to obstruction of the tube or failure to obtain proper duodenal and gastric contents from the tubes. Direct comparison could not be obtained therefore between the results under the two conditions. Although the successful test in each case well agreed with the mean results of the corresponding groups, these results are not included in the data.

The other results are listed in table 1 showing the output of pancreatic juice as well as the bicarbonate, trypsin and amylase contents in the control experiments. The values are presented as such without making the time correction for the loss of potency of the secretin preparation.

Table 1.

Control experments. The pancreatic secretion after intravenous injection of secretin (1 clin. unit/kg. body wt.). Collection period 60 minutes.

Sub- ject	Volume cc.	Bicarbonate cc. N/10	Amylase units	Trypsin units
O.P.	184	176	504	62
E.S.	163	149	576	95
P.V.	139	137	535	62
O.K.	155	162	553	83
N.K.	255	270	1077	148
E.H.	147	142	461	69
H.T.	160	147	561	91
R.H.	188	165	544	68
E.L.	155	150	456	63
H.K.	145	133	499	74

According to the large material of LAGERLÖF (1942) the mean volume decrease on account of the inactivation of the secretin preparation was 0.080 ± 0.023 cc. per day.

Since the control and anoxia experiments in each subject were made within a few days or weeks of each other, the effect of such short periods could be neglected in the calculations.

For the purpose of comparison the means of the present control material are listed together with the normal results of Lagerlöf's larger material in table 2. As can be seen in this table the writers average results are slightly lower than those of Lagerlöf's material, although they still fall well within the range of his variations. The most possible explanation for this difference in yield, to the writer's understanding is the difference

in the way of maintaining the suction in the tubes. It is quite certain that with the syringe method the pressure could not be kept so constant as by using other devices. During the period of changing the syringe there might also be a chance of the juice escaping from the tube.

Table 2.

Comparison of the control results by Lagerlöf and the present material.

	Lagerlöf's material		Present material		% difference
	Mean	St.D	Mean	St.D	
Volume cc.	206 $\pm$ 3.6	29.4 $\pm$ 2.5	169 $\pm$ 10.7	34 $\pm$ 7.5	— 18
Bicarbonate cc. N/10	196.9 $\pm$ 4.6	37.9 $\pm$ 3.3	163 $\pm$ 12.3	40 $\pm$ 8.9	— 17
Amylase units	637 $\pm$ 19	218 $\pm$ 27	577 $\pm$ 51.2	180 $\pm$ 42.4	— 15
Trypsin units	86.2 $\pm$ 4	28.1 $\pm$ 2.9	82 $\pm$ 8.2	26 $\pm$ 5.8	— 4

Otherwise the present results agree with Lagerlöf's material as far as the general type of secretory curves are concerned. The rate of secretion is the greatest during the first and second ten minute collection periods after the secretin administration. The enzyme contents are also greatest during this period indicating that the enzyme material present in the pancreas is washed out.

The presented material is rather homogeneous. Only one of the test subjects (N.K.) showed a distinct difference in the amount of juice secreted by his pancreas. The only female (H.T.) included in the material showed no difference from the general trend so that it was felt justified to include her results together with the other material.

Anoxia experiments

Actually, two different degrees of anoxia were tested. The highest one corresponds to an altitude of 5500 meters and the other to 3500 and 4000 meters.

Table 3 represents the results obtained at the different levels of simulated altitude. By comparing the average total output of the juice secreted during the anoxia test with that of the control test it becomes evident that the former is distinctively smaller. The difference between the corresponding means is statistically significant ($P < 0.01$).

Table 3.

Anoxia experiments. The yield of duodenal drainage after intravenous secretin injection. Collection period 60 minutes.

Sub- ject	Volume cc.	Bicarbonate cc. N/10	Amylase units	Trypsin units	Simulated altitude
O.P.	66	59	216	38	5500 m.
E.S.	52	37	214	32	» »
P.V.	42	34	196	28	» »
O.K.	41	66	227	27	» »
N.K.	98	84	423	55	» »
E.H.	64	60	220	22	4000 »
H.T.	85	72	331	43	4000 »
R.H.	80	55	288	29	4000 »
E.L.	90	88	247	33	3500 »
H.K.	73	60	260	37	3500 »

The corresponding amounts of the analysed constituents as well as the total output of juice in control and anoxia tests are presented in figure 1. From this figure it can be noticed that all of the constituents are reduced approximately to the same extent.

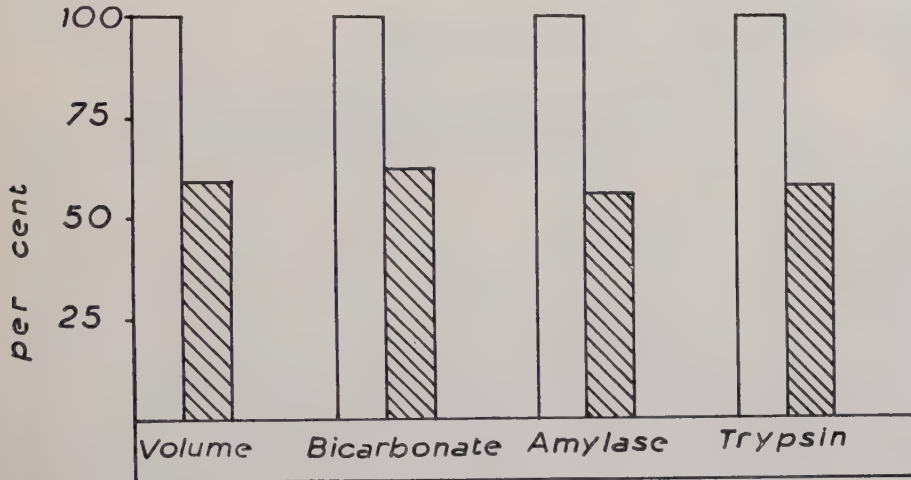


Figure 1. Comparison of the pancreatic output at normal and reduced atmospheric pressure. Each column presents the total yield obtained during a 60 minute collection of duodenal juice after an injection of a standard dose of secretin. The striated area represents the anoxia results.

Figures 2 and 3 present the comparison of the rates of volume and amylase secretion at normal atmospheric and simulated altitude conditions.

It is interesting to find a slight difference in appearance of the enzyme secretory curves obtained in the two conditions. Whereas in the control curve there is a slight increase in the secretory rate of the enzymes towards the end of the collection period, no such increase can be noticed in the altitude test. The enzyme output gradually diminishes towards the end of the collection.

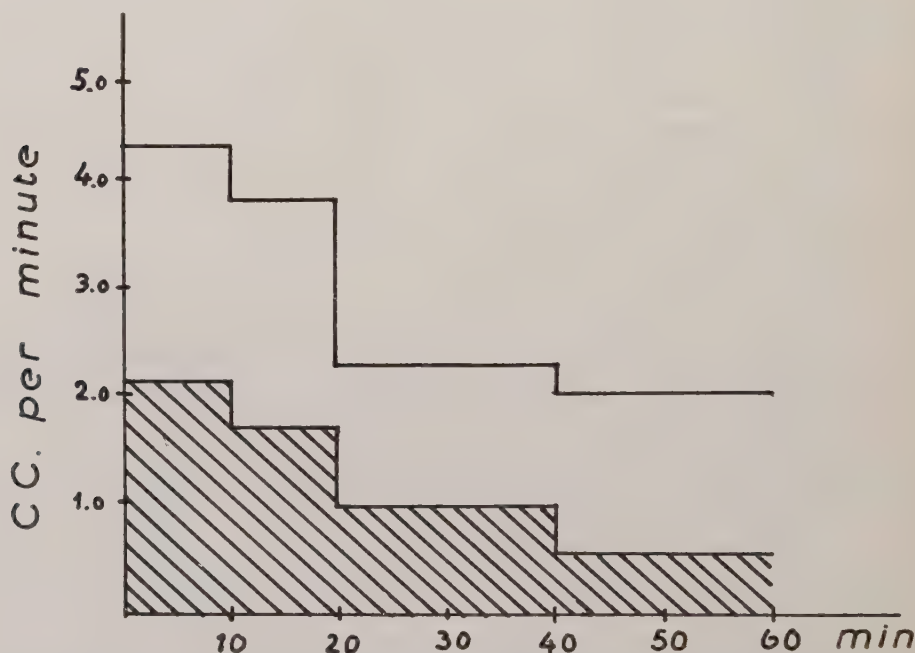


Figure 2. Comparison of the secretory rate in control and anoxia experiments. The upper line presents the mean response of 10 subjects to the administered secretin at atmospheric pressure. The lower line presents the mean response to the same dose of secretin at reduced atmospheric pressure corresponding to simulated altitudes of from 3500 meters to 5500 meters.

In table 4 the results obtained at the two different altitude levels are treated separately. On account of the smallness of the material the results at 3500 and 4000 meters are combined since the difference of 500 meters corresponding to a change of c. 9 mm. in the oxygen partial pressure in the air is not very great.

As can be seen from table 4 there is a slight difference in the means of the two groups although this difference is in no way statistically significant. The »hypersecretor» (N.K.) happens to belong to the group treated with the higher degree of anoxia. In a small material like this, his results, as far as the absolute values are concerned, may mask the real

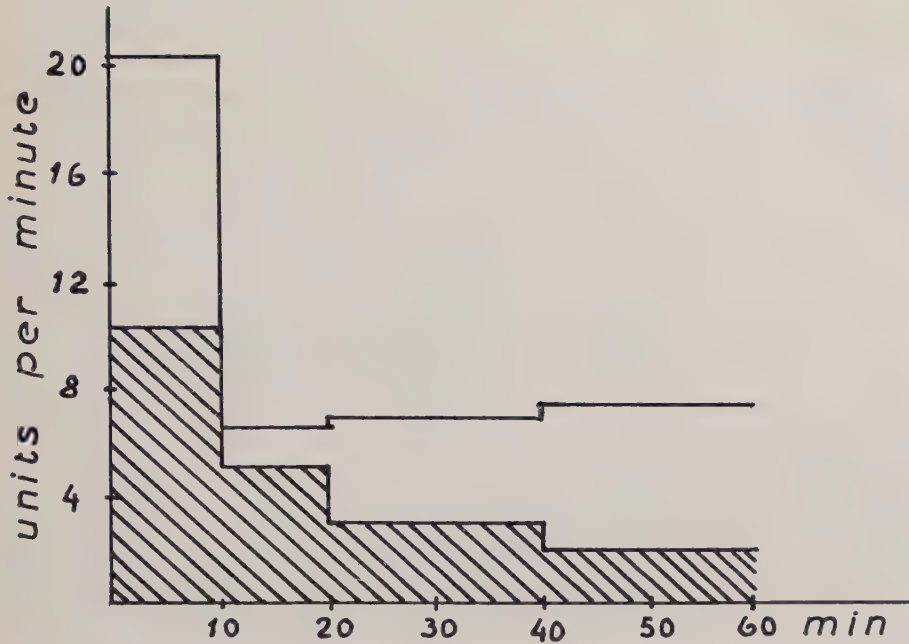


Figure 3. Comparison of the amylase output in control and anoxia experiments. The upper line presents the mean response of 10 subjects to the administered secretin at atmospheric pressure. The lower line presents the mean response to the same dose of secretin at reduced atmospheric pressure corresponding to simulated altitudes of from 3500 to 5500 meters.

effect of the different altitude levels by causing an elevation in the mean values of the group. In order to elucidate the problem further the percentage reduction of the output was calculated. These values can be

Table 4.

Comparison of the mean values of pancreatic output at different degrees of anoxia.

	5500 m		3500—400 m			
	Mean	St.D	Mean	St.D	<i>t</i> values for the means	P
Volume cc.	60	23.6	78	10.2	1.61	.1 — .2
Bicarbonate cc. N/10	56	20.8	67	13.3	1.05	.3 — .4
Amylase units	295	104.4	269	42.3	0.58	.5 — .6
Trypsin units	36	7.8	33	7.9	0.63	.5 — .6

seen in table 5. When using this criterion it becomes evident that there is a statistically significant difference in the reduction of both volume and bicarbonate output at the various levels of simulated altitude. The reduction is greater at the higher degrees of anoxia.

Table 5.

Comparison of the percentage reduction of the pancreatic secretion at different degrees of anoxia.

	5500 m. Mean reduction from control %	3500—4000 m. Mean reduction from control %	t values for the means	P n = 8
Volume	67	50	4.80	< 0.01
Bicarbonate	68	54	2.6	< 0.01
Amylase	60	46	7.12	0.05 — 0.02
Trypsin	57	55	0.32	.7 — .8

It appears to be of particular interest that also the alkali output by the pancreas is diminished by oxygen lack.

Blood sugar

Table 6.

Blood sugar before and immediately after the exposure to simulated altitude.

Subject	Before anoxia mg %	After anoxia mg %	Difference %	Simul. alt. Meters
H.T.	95	114	20	3500
R.H.	98	111	13	»
E.K.	105	117	11	»
O.K.	92	108	18	5500
N.K.	108	122	13	»
O.P.	91	98	7	»
E.S.	85	97	14	»
Mean	96	109	14.5	

It can be seen from table 6 that a slight tendency towards increased blood sugar values appeared during anoxia. The increase, however, is very small the range of the percentage difference between the values before and after anoxia being 7 to 20 per cent. The absolute values for the blood sugar after anoxia ranged from 97 mg % to 122 mg %.

Alkalosis experiments

The effect of the alkali excess was studied in 5 persons. The amount of alkali ingested by each subject was 36 grams as previously described. The effect of the drug was determined by analyses of the alveolar carbon dioxide content and urinary pH. This effect can be seen from tables 7 and 8. It can be seen that the administration of bicarbonate resulted in a constant increase on the alveolar carbon dioxide content and elevated urinary pH in each subject. It should be noted that the first values for the alveolar carbon dioxide in table 7 are obtained already after ingestion of the alkalinizing salt and as such are higher than usually found in normal subjects (HALDANE and PRIESTLEY, 1905). There was, however, still an elevation of these values amounting to an average percentage increase of 0.5 per cent. The urinary pH was in all cases above 8.0. Whereas the urinary pH remained elevated several hours after the last tablets of bicarbonate were taken, the alveolar carbon dioxide percentage usually decreased in about two hours.

Table 7.

Percentage alveolar carbon dioxide content during the alkalosis experiments.

Sub- ject	Alveolar CO ₂ -percentage		
	Approximately 3 hours before secretin test	30 min. before secretin test	Immediately after secretin test
H.T.	6.06	6.91	6.27
O.K.	6.30	6.80	6.59
N.K.	6.48	6.97	6.75
R.H.	6.72	7.07	6.18
O.P.	6.05	6.63	6.23

Table 8.

Urinary pH-determinations for the alkalosis experiments.

Sub- ject	The night before secretin test		Relation to secretin test			
	7 pm	10 pm	3 hours before	1 hour before	Immediately before	After
H.T.	5.26	5.89	6.23	7.04	8.09	8.05
O.K.		7.14	7.20	7.25	8.18	8.12
N.K.	5.65	7.55	6.48	7.14	8.10	8.13
R.H.	6.50	6.84	6.88	7.90	8.14	8.15
O.P.	6.76	7.04	7.16	7.44	8.01	8.00

The secretin test was performed at the peak of the drug influence. In each case it was completed while the urinary pH still was elevated. The experiments are therefore considered to present the pancreatic function during uncompensated alkali excess conditions in blood.

Table 9.

Alkalosis experiments. The volume and bicarbonate output after intravenous injection of secretin. Collection period 60 minutes divided into 4 parts.

Sub- ject	Volume cc.						Bicarbonate cc. N/10					
	During alkalosis					Control	During alkalosis					Control
	10 ¹	10 ²	20 ¹	20 ²	Total	Total	10 ¹	10 ²	20 ¹	20 ²	Total	Total
H.T.	56	44	41	35	176	160	69	48	36	28	181	147
O.K.	58	45	50	42	195	155	64	51	48	41	204	162
N.K.	70	49	59	63	241	255	77	53	60	61	251	270
R.H.	56	42	68	46	212	188	61	44	61	42	208	165
O.P.	48	48	62	52	210	184	53	57	56	49	215	176
Mean	207					188	212					184
t	0.96						1.10					
P	.3 — .4						.2 — .3					

The results of the secretin test are shown in table 9. There appears to be a general increase in the volume output as well as bicarbonate secretion under the conditions. On the other hand the enzyme output is not increased as indicated by the data on the amylase output (table 10).

Table 10.

Alkalosis experiments. The amylase output after intravenous injection of secretin. Collection period 60 minutes divided into 4 parts.

Sub- ject	Amylase units					
	During alkalosis					Total
	10 ¹	10 ²	20 ¹	20 ²	Total	Control
H.T.	191	59	121	132	503	561
O.K.	251	89	126	99	565	553
N.K.	301	118	185	215	819	1077
R.H.	270	78	115	66	529	544
O.P.	187	121	87	96	491	504
Mean	581					648
t	0.13					
P	.9					

These changes appear, however, not to be statistically significant.

Chapter III Animal experiments

Methods

Collection of the pancreatic juice

It has been credited to GRAF in 1662 to be first to insert a cannula into the pancreatic duct in dogs and to collect juice from the gland directly. Ever since such cannulation has served for experimental purposes in acute type of studies, and the procedure has remained the same throughout the years. The same procedure was also employed in this work.

Operative procedure

All of the animal experiments were performed on mongrel dogs, under light sodium pentobarbital anesthesia, the initial dose of which being 40 mg. per kg. of body weight. The dogs were fasted at least 15 hours before the operation. The weight of the animals ranged between 8.4 — 11.0 kilos.

After a middle line incision of the abdomen the pancreas was carefully exposed and the main duct was cannulated with a thin glass cannula.

This duct lies in general about 2.5 cm. craniad from the division of the distal part of the pancreas from the duodenum on to which the other part of the gland is attached. After ligation of the small branch of blood vessels at this point the light duct is in general easily visible under the serosal coat of the intestine. The cannula is inserted into the duct near its entrance into the duodenum and fixed in position by a silk thread. The thread is introduced under the duct by using a small curved needle. The cannula now very soon usually fills up with clear juice as an indication of a successful procedure. The first drops are thick and according to their appearance perhaps contain mucus secreted by the epithelium of the duct. In case of obstruction by blood or by this thick secrete it is a good practice to first inject water with a syringe and long needle into the cannula and also give the animal a strong secretin stimulus to have the duct and cannula washed out.

It has been first demonstrated by HESS (1907) that in dog there are not so infrequently accessory ducts in addition to the main duct and the one accompanying the common bile duct. Care was taken to ligate all these accessory ducts if found. The most common location of the third duct is about 1 — 2 cm. craniad from the main duct. It was also found to exist in the dogs operated for this work in about one third of the animals. The accessory duct entering the duodenum with the common bile duct was

excluded by placing sutures around the terminal portion of the common duct.

The pylorus was clamped in order to secure the avoidance of an additional stimulus by means of allowing the HCl to escape into the duodenum. The forceps used for the purpose were covered by a rubber tubing in order to avoid unnecessary injury to the area. Care was also taken that the blood vessels running over this area to supply the pancreas were not severed.

Stimulus applied

In these animal experiments a constant and continuous stimulus was employed. This was achieved by injection of a secretin-pancreozymin solution into the femoral vein by means of a constant infusion pump. The rate of infusion was so fixed that the secretion of the gland was approximately half of its maximal capacity. This was first determined by introducing a high dose of secretin.

The secretin used in this work was prepared by the method of KRUP (1948) and also kindly supplied by him. Due to the high potency of the preparation for secretin and small amounts of panereozymin present in it another preparation containing more panereozymin was added to the solution in order to have a stimulus both for the ecboic and hydrelatic functions of the pancreas. The concentrations of secretin and panereozymin in the administered solution were kept constant and only the rate of infusion was changed to achieve the desired control secretory rate of the pancreas. The infusion rate ranged from 1.1 to 1.55 cc. per minute.

The amount of hormone concentrates used for making the solution were 50 mg. of the secretin preparation and 20 mg. of panereozymin preparation dissolved in 400 cc. of normal saline.

Method for producing anoxia

The anoxia effect was produced by having the animal breathe from a Douglas sack containing a fixed concentration of oxygen-nitrogen mixture. The expired air was conducted by means of valves out of the system so that no accumulation of carbon dioxide took place. The tube attached to the gas container could be connected to the cannula which had been inserted into the trachea of the animal. By means of a T-tube with a large diameter and by using suitable valves linked to the system the animal could

be kept breathing either from the prepared gas mixture or from the room. The tubes and additional arrangements described above were so made that they did not offer a different resistance for the respiratory movements when the animal was breathing room air or from the Douglas sack.

It should also be noted that the shift of the animal to anoxic conditions could be done without any other manipulation than the mentioned opening and closing of the proper connections.

After a few experimental trials the oxygen content of the gas mixture was fixed to 8 per cent. This kind of mixture was thereafter used in all of the experiments. The correct concentrations of the oxygen and nitrogen mixture was attempted by measuring the quantities of each gas brought into the sack. This was done by attaching a gas meter to the cylinders from which the pure gases were taken. The conditions did not allow more accurate analyses of the gas mixture.

Experimental procedures

The dog was allowed to recover from the operative manipulations for an hour during which period the constant infusion of the secretin stimulus was started. All of the juice secreted during this period was discarded and only the rate of secretion was observed. The constant secretin stimulus now had »washed out» the gland from its restored enzyme material and the rate of secretion had reached usually a constant level. After this, three subsequent samples collected over a period of 10 minutes were taken. These samples represented the control period. The volume of these samples had not to differ more than 10 per cent from each other. If they did, the collection was continued until this constancy of the secretion was obtained. After the control secretion had now been established the animal was made to breathe from the gas mixture. The collection was continued as before. The anoxic exposure was fixed to 13 minutes in each experiment, the length of the exposure being determined by the capacity of the gas container.

The samples collected during the anoxia period were divided into three portions corresponding to the juice secreted during the first and second five-minute periods of anoxic exposure, the last three minutes comprising the last portion.

After releasing the animal to breathe normal air again the initial recovery period was divided into two five-minute periods and thereon into ten-minute periods.

The samples which had been collected into graduated centrifuge tubes were immediately placed in a refrigerator.

Analyses on the juice

B i c a r b o n a t e

The bicarbonate determinations were performed as described in the first part of the work.

A m y l a s e

A different method from that used in the human experimentation was adopted for the amylase determinations. This change was made mainly on account of the availability of the equipments for the employed modification of the WILLSTÄTTER method as it has been described by SCHMIDT, GREENGARD and IVY (1934), and which modification had gained general acceptance in the laboratories where this study was made.

This method also applies starch as a substrate but differs from the LAGERLÖF method used in the first part of the work in that the actual amount of maltose liberated during the hydrolysis is determined by estimating the amount of iodine taken up by the maltose according to the following reactions.



The results may be expressed as WILLSTÄTTER *units* based on the same principle as described for the LAGERLÖF units, or simply in the amounts of mg. of maltose formed during the hydrolysis under the given conditions. Since 0.1 n iodine is equivalent to 17.15 mg. maltose, the units used in this study are calculated from the following equation

$$\frac{(c - s) \left(\frac{\text{normality of KI used}}{0.1 \text{ n}} \right) \times 17.15}{\frac{\text{normality of KI used}}{\text{normality of Na}_2\text{S}_2\text{O}_3}} \quad \text{or in the case}$$

normality of the iodine solution is 0.1

$$(c - s) \times \text{N. of Na}_2\text{S}_2\text{O}_3 \times 171.5$$

$s = \text{cc. of Na}_2\text{S}_2\text{O}_3$
used for control blank

$c = \text{cc. of Na}_2\text{S}_2\text{O}_3$
used by the sample.

Procedure

The whole procedure is as follows. 15 cc. of a 2% soluble starch solution buffered to pH 6.8 using a 0.2 M phosphate buffer is pipetted into 250 cc. Erlenmeyer flasks which are then warmed up to 37°C in a constant water bath. It is practical to have a heavy metal ring around the flasks in order to keep them standing in the water. The enzyme solution, in a dilution 1:25 parts in water, is then added into the substrate. The flasks are then removed exactly 15 minutes after the addition of the enzyme solution into them and the hydrolysis is stopped by adding 2 cc. of n. HCl into the mixture. Exactly 20 cc. of N/10 KI is added followed by 50 cc. of N/8 NaOH. The flasks are well shaken during the adding and more NaOH is added if the contents in the flask do not fade. After 15 minutes, at which time the contents still should be colorless, sufficient amount of dilute H₂SO₄ (about 2 cc. of 10 % solution) is added to make the mixture acid. The excess iodine is then titrated with 0.05 N sodium thiosulfate. A blank is set up containing the same amounts of reagents, but instead of adding active enzyme solution, it is first boiled in order to destroy this activity.

Blood sugar

Samples for the blood sugar determinations were taken from the femoral vein and analysed according to the method of HAGEDORN-JENSEN.

Results

Volume and bicarbonate output

In all, twenty dogs were used in this part of the work. Seven of them were, however, discarded on account of inconsistency of the basal secretion. The experiments of the remaining 13 were completed and are reported.

As usually is the case in animal experimentation, a great variation in the sensitivity of the animals towards the applied standard stimulus was observed. The range of the secretory rate was 0.12—0.44 cc. per minute during the control period.

In the first experimental trials it was observed that during the first minutes after the animal was exposed to oxygen lack, the secretory rate had increased. Occasionally this increase was of such a magnitude that the total amount of juice when determined from ten minute collections masked the decrease which occurred later in this period. Although the five minute

point does not necessarily mean the exact culmination for the changes in the secretory rates, it was chosen in order to detect the consistence of the phenomenon without losing the chance of obtaining samples large enough to be subjected to the chemical analyses.

The length of the exposure to anoxia was fixed to thirteen minutes. After this the recovery period began. The first ten minute period of this was also divided into two five minute intervals. Since the amount of juice secreted for these samples was usually too small to allow individual analyses of them, they were combined for this purpose. For the same reason the last three minute samples collected during anoxia were occasionally analysed with the previous five minute samples.

Figure 4 shows the mean of the secretory rate for the whole material in these experiments.

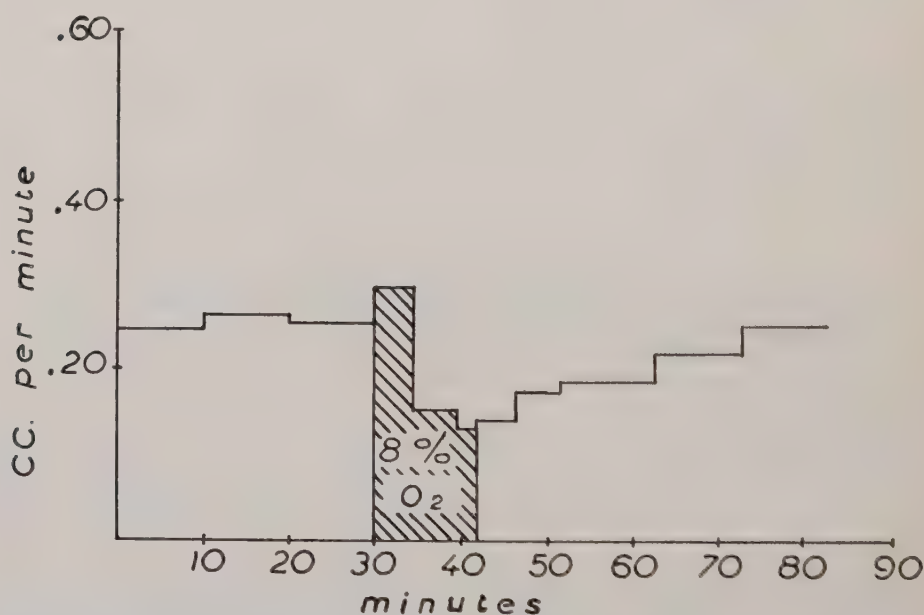


Figure 4. Animal experiments, volume output. The graph represents the mean rate of secretion during the constant secretin-pancreozymin stimulation. The striated area presents the anoxia period.

It can be seen from this illustration that the anoxia caused a reduction of the pancreatic secretion also in dog. There is no point in trying to compare the sensitivity of man and dog in this respect. The presented material does not entitle one to make such evaluations owing to the fact that the experimental conditions varied so radically from each other in the two types of experimentations. To mention perhaps one of the most

important differences, the human experiments were performed in intact subjects whereas the animal experiments were done after surgical manipulations and under anesthesia.

The mean output of bicarbonate is shown graphically in figure 5. As can be seen from this illustration a small peak occurs in the output corresponding to the first five minute period of the breathing of oxygen-poor air. The increase in the bicarbonate output is by no means a constant phenomenon. It occurred however in 9 out of the 13 experiments. The mean values for the basal secretion and the first five minute values during anoxia do not differ statistically ($.3 < P < .4$). The bicarbonate output reduction after this point is however also statistically significant ($P < 0.01$).

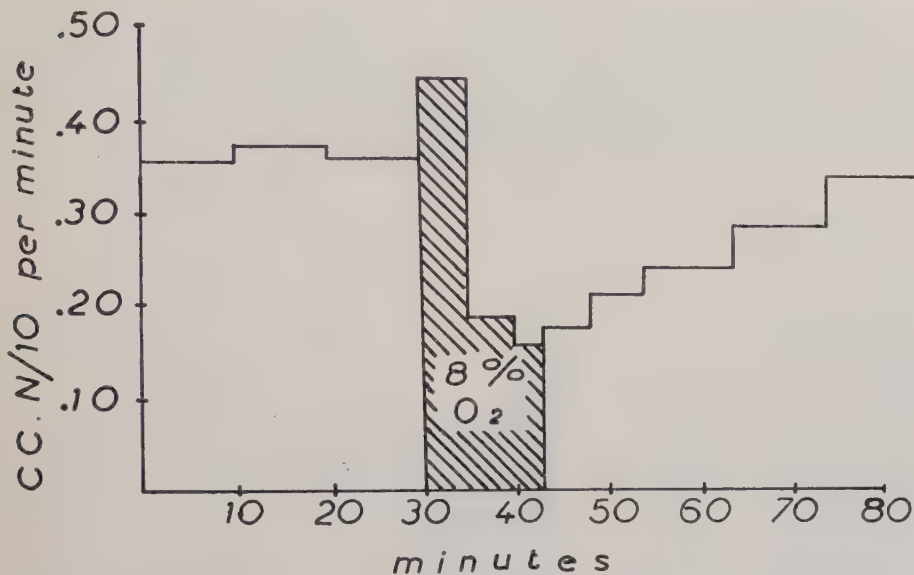


Figure 5. Animal experiments, bicarbonate output. The graph represents the mean rate of bicarbonate output during the constant secretin-pancreozymin stimulation. The striated area presents the anoxia period.

While performing the tests, it was thought that the increase of respiratory movements which occurred during the onset of anoxia, might have exerted some kind of mechanical massage on the pancreas. In order to study this possibility the four last dogs in the series were treated differently in the operative procedures. In spite of leaving the pancreas only partly exposed through the incision wound it was taken completely out of the abdominal cavity together with the corresponding duodenal loop. The pancreas with the attached duodenum was now placed on the outer surface of the body. No massage effect by the surrounding tissues was now possible.

The exposed pancreas and intestine were kept moist by placing cheese cloth dipped into normal saline solution on it.

The results obtained after this modification did not differ from those which were obtained by the original procedure. The peak occurred in three dogs out of four thus indicating that some cause other than direct mechanical massage on the pancreas must be held responsible for the phenomenon.

Amylase secretion

The amylase output is illustrated by the figure 6. It is seen that a peak similar to that observed in the bicarbonate secretion did not occur in the amylase output. The reduction of the secretory rate is also much greater as regards the volume or bicarbonate values, being in the case of amylase from 469 units to 123 units in average.

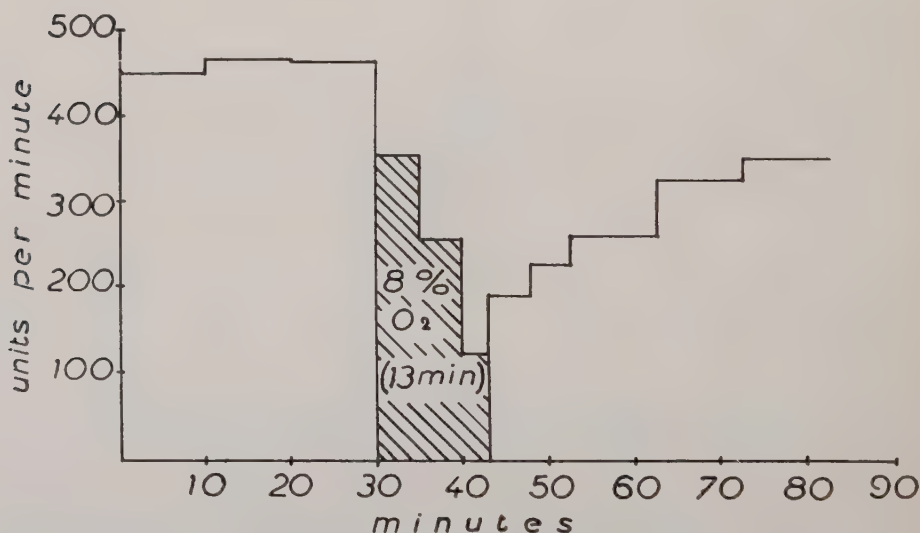


Figure 6. Animal experiments, amylase output. The graph represents the mean rate of amylase output during the constant secretin-pancreozymin stimulation. The striated area presents the anoxia period.

It is also interesting that whereas the volume and bicarbonate secretion had gained the control level in about 40 minutes after the breathing of normal air, the amylase output at this time still was some 20 per cent below the control level.

Blood sugar

The blood sugar determinations in the human material were made from samples taken not during the exposure to oxygen lack but only immediately after this. In order to obtain more reliable view of the possible changes in the blood sugar during anoxia such determinations within short intervals were performed on three dogs throughout the experiments.

One typical experiment performed as described is illustrated by the figure 7. It can be seen that the secretory rate of the juice as well as the enzyme output does not parallel the course of the blood sugar curve. The same trend was also obvious in the two other similar experiments.

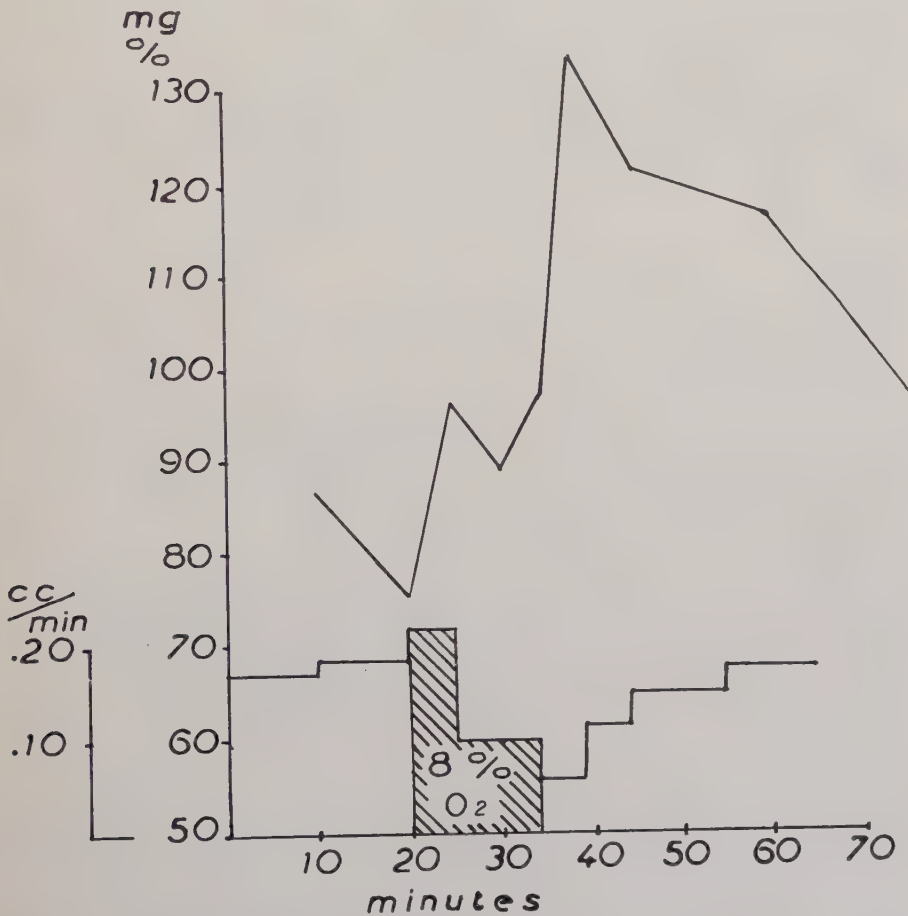


Figure 7. Simultaneous determinations of blood sugar and pancreatic secretion in anoxia test. The upper curve represents the blood sugar and lower curve the rate of pancreatic secretion in one experiment.

These observations discouraged the writer from continuing the blood sugar determinations in order to be able to find in these changes a causative factor for the altered secretory behaviour of the pancreas during anoxia.

Chapter IV General discussion

Evaluation of the results

From the reported data it is evident that the pancreatic function test when performed under anoxic conditions yields smaller values than when performed at normal atmospheric pressure. This statement holds true both for the hydrelatic as well as ecobolic function of the gland. In this respect the results differ from those reported by RASENKOW who claimed that the duodenal drainage yielded more bicarbonate at high altitudes. The lowest degree of altitude corresponded to 3500 meters in the present work. Whether the results are different below this point was not established in this study. It should also be pointed out that the present investigation was made under anoxic conditions of s h o r t d u r a t i o n.

As shown by the animal experiments, the bicarbonate output is frequently increased during the first minutes after the exposure to anoxia. This effect is rapidly followed, however, by a great suppression of the secretion so that the net result after a few minutes is a greatly reduced secretion.

With our present knowledge it is not possible to say whether the enzyme producing elements are different from those producing the juice and inorganic constituents of the pancreatic fluid. Suggestions have been made that these two main components have a different origin (GROSSMAN and IVY 1946) since the observation was made that the degeneration of the pancreatic ductule cells as it occurs in alloxan diabetes is associated with a diminution in the volume of juice secreted in response to secretin. This observation has been offered as an indication that the ductule cells might be the site where secretin acts to stimulate secretion of water and bicarbonate. The granular formation of the acinar cells is generally accepted as being associated with the enzyme production of these cells.

Regardless of whether the site of enzyme and bicarbonate secretion in the gland is the same or not, the results in the present work seem to indicate that these productions are not similarly affected by anoxia. The following observed facts may be taken as an evidence to support this statement.

Although the ecobolic and hydrelatic functions especially in the human experimentation would suggest that they are equally suppressed by anoxia this may not be so, and the results obtained would not give the real view of the actual process. As pointed out, the secretin test is a washout test.

In this work it means that the enzyme constituents obtained after the secretin stimulation as performed during anoxia could hence be performed in the gland before the test was begun. It is not possible by a single test to determine how much the actual synthesis of enzymes was affected by anoxia. This point was realized too late to allow more material to be collected to elucidate this effect. This was one reason to study the effect on dog. The animal experiments seem to give the required answer. The test when using the constant secretin and pancreozymin stimulation and after establishing a relatively constant basal secretion and after establishing a relatively constant basal secretion is suitable for detecting changes separately in the ecbolic and hydrelatic function.

Figures 5 and 6 presented the mean secretory rate of bicarbonate and amylase for the whole animal material. Anoxia initially effects both the functions to the same extent. However, the recovery of the gland after the exposure shows a difference in the rate of bicarbonate and amylase secretion. It would appear that the recovery of the enzyme production is slower than that of the bicarbonate and would therefore suggest that either the site of the formation of these constituents is different or that processes perhaps behave independently of each other.

From the results analysed so far it may be possible to conclude that the pancreatic secretion is greatly suppressed by anoxia and that the results would also supply evidence that the recovery takes a longer period for the enzyme producing elements than for the water and bicarbonate formation.

Mechanism of the anoxic suppression of pancreatic secretion

Lack of oxygen per se

As mentioned in the classification of different types of anoxic conditions, the anoxic type of anoxia results in a true reduction of the arterial oxygen saturation. The level of the oxygen saturation depends mainly on the degree of the changes in the oxygen pressure to which the subject is exposed.

For our purpose it is interesting to know that the changes in the arterial oxygen saturation take place relatively soon. In a review on the subject HENSON, GOLDMAN, CATCHPOLE, VOLLMER, KING and WHALEY (1947) have determined the average figures given by various investigators on the oxygen saturation at different levels of simulated altitude. From these data it appears that the average values for the oxygen saturation in arterial blood at the altitude used in our experiments mainly at 3500 and 5500 m. would be 80 per cent and 72 per cent oxygen saturation in the same order.

From the same analyses it also appears that the values obtained after the first 10 minute exposure are higher than for the value obtained after 15—20 minutes. However, the values after one hour did not show any significant deviation from those taken between 15—30 minutes.

These analyses would therefore indicate that the present pancreatic function tests were performed under a rather constant degree of anoxia.

This same statement applies also to the animal experiments according to a recent report by GOLLWITZER-MEIER (1947) since breathing of 8—9.5 per cent oxygen with nitrogen results in a very rapid decrease in the oxygen saturation. In some of his experiments the steep fall in the oxygen saturation could be demonstrated in 40—45 seconds after the exposure to the anoxia. The determinations were made by direct oximeter recordings on dogs, such procedures allowing a rapid detection of changes in the oxygen saturation.

When considering the various factors which during anoxia might contribute to the suppression of the pancreatic function the first to come in mind is thus the lack of oxygen per se in the blood supplying the gland. The activity of the pancreas is associated with increased oxygen uptake as previous studies have demonstrated (GERARD and STILL 1933, ÅGREN 1934, DEUTSCH and RAPER 1936). For the moment it would be interesting to know whether such studies would reveal any difference in the oxygen requirements for the enzyme formation on the other hand, as compared to the maintenance of the hydrelatic function. Unfortunately no such information is at hand. In vitro it has been shown that both pancreozymin and secretin increase the oxygen uptake by the pancreas. (DAVIES, HARPER and MACKAY (1949).

As to the relationship between the arterial oxygen pressure and the pancreatic secretory capacity the present studies do not offer any exact figures. The arterial oxygen contents were not analysed and the whole experimentation was based on the assumption that under the given conditions an anoxemic condition was also produced.

Therefore, it can only be said that among the factors leading to the reduction of the pancreatic function, the lack of oxygen in blood as such may be one.

Anoxia results also in some other changes in the blood. As mentioned before, anoxia may be accompanied by a disturbed acid base balance in the blood as well as by changes in the electrolyte contents.

Changes in the acid base equilibrium

The control studies made in this work rule out the possibility that relative alkalosis under the conditions could actually explain the diminished

secretion by the pancreas. As a matter of fact real alkali excess results in an increased alkali output by the pancreas and although the enzyme production perhaps is a little depressed during these conditions this effect results in much less change in the enzyme output than when anoxia is applied.

A better way to study the effect of anoxia alkalosis would be to produce similar changes in the acid base conditions by allowing the subject to hyperventilate for a certain period of time. Although this difficult procedure was not employed, the animal experimentation would suggest that hyperventilation really results in an increased alkali output than in a reduction. The increased secretion of juice and of bicarbonate which occurred in these experiments frequently during the first minutes after the anoxic exposure are interpreted as being a result of the hyperventilation.

Changes in the electrolyte contents of blood

Although evidence is available indicating that anoxic anoxia is associated with changes in blood potassium (HALL, DILL and BARRON, 1936, MACQUARRIE, ZIEGLER, STONE, WANGESTEEN and DENNIS 1940) as well as calcium contents (DUGGE 1926 and GORALEWSKI 1937) no information, on the other hand, is available as regards the relationship between these electrolytes and the pancreatic secretion. Only after such relationships have been established will it be possible to postulate the significance of the changes in the blood potassium or calcium to the observed decrease of the pancreatic secretion during anoxia.

Changes in the nervous system

Although the central nervous system is greatly affected by anoxia these effects perhaps do not as such bear any significant relationship to the problem to be studied in this work.

It would appear to be of more importance to study the data of the effects of anoxia on the autonomic nervous system since it is these nervous routes along which the effects of anoxia would be conveyed to the pancreas.

Furthermore, since both the sympathetic and parasympathetic components of the autonomic nervous system exert their regulatory effects on the external pancreatic secretion it is appropriate to discuss separately each of these components.

Sympathetic system

As it will be pointed out later in the discussion the release of adrenaline by the adrenal glands has been claimed to be augmented by anoxia. Since this secretion is entirely under sympathetic control it would suggest a stimulatory effect of anoxia on the sympathetic nervous system.

Other experimental evidence to support such claims has also been presented. (GELLHORN and LAMBERT 1939, GELLHORN, CORTELL and CARLSON 1942).

Gellhorn and his co-workers in a series of experiments dealing with autonomic and somatic responses elicited by stimulation of hypothalamus, medulla and spinal cord on narcotized and decerebrate cats suggested that the autonomic centers in the central nervous system are less sensitive to anoxia than are the somatic centers. The vasomotor and respiratory centers are intermediate between the somatic centers. The authors assume that the increased excitability of the autonomic centers greatly contributes to the resistance of the organism to anoxia. The increased sympathetic activity could exert its effect on the pancreas in several ways.

One possibility is that there is a direct effect of the sympathetic system on the pancreas via the nervous fibres with which the gland is supplied.

Since it is generally accepted that the sympathetic control of the pancreas is maintained through the constrictor action of these fibres on the blood vessels supplying the gland, the effect of sympathetic over-activity can be considered in the light of the general rearrangements of the circulation during anoxia. This will be discussed later.

The third possibility, the probable increased adrenaline discharge by the adrenal glands will be discussed also later.

Parasympathetic system

Very little if any work has been done to study the effect of anoxia on the sacral component of the parasympathetic system.

As to the cranial portion YOSOMIYA (1927) claimed an increased sensitivity to electrical stimulus of the peripheral vagus as indicated by studies on the heart rate in rabbits after stimulation of the cervical vagus trunk.

LOEWY and HELLER (1933) also pointed out the altered reactivity of the autonomic system under anoxia. This was demonstrated by testing the effect of parasympathicomimetic drugs on the intestinal motility and salivary secretion during anoxia.

Other similar types of reports have been offered (VAN LIERE and CRISLER 1933, BORGARD 1934).

That the sympathetic stimulatory effect of anoxia might be dominant in the earliest phases of anoxia has been first suggested by HIMWICH, MARTIN, ALEXANDER and FAZEKA (1939) who were studying the effects on dog and analysing the cardiac function and blood sugar. Performing such studies under normal conditions and after vagotomy, stellate ganglionectomy and splanchnic resection their conclusions were that the initial sympathetic influence was followed by a parasympathetic activity. Similar suggestions have received experimental support by the studies made by MCQUARRIE, ZIEGLER, STONE, WANGENSTÉEN and DENNIS (1939), FELDMAN, CORTELL and GELLHORN (1940) as well as by MCQUARRIE, ZIEGLER and HAY (1942). The studies reported by these investigators have convinced the authors to conclude that the vago-insulin system is stimulated by anoxia.

The parasympathetic stimulation caused by anoxia, however, appears to be masked in a normal animal by the dominating effect on the part of the increased activity of the sympathetic system.

The possible over-activity of the parasympathetic nervous system referred to in the preceeding discussion might also have occurred in our experiments. The possible role of this in the results cannot, however, be valuated on the basis of the present experiments. The results show no increase in the enzyme production which could be taken as an indication of a stimulatory effect carried to the gland via the vagi. If, on the other hand, the vagal inhibitory effects had occurred, one would expect that the possible constriction of the glandular ductlets would have been followed during the recovery period in the animal experimentation by an increased discharge of accumulated material and especially of enzyme material. No such effect could, however, be noticed.

Changes in the adrenal function

Effect of anoxia on adrenaline secretion

The role of the adrenal glands also in the stress produced by anoxia has drawn the attention of the investigators. It has been demonstrated that the tolerance of adrenalectomized animals towards anoxia is considerably reduced as compared to normal animals (CANNON 1919). Both the medullary and cortical functions under these conditions have been treated with experimental analyses.

Since the relationship between the external pancreatic function and the adrenal cortical functions have not so far been established, it suffices here only to discuss the medullary activity of the adrenals.

Owing to the methodical difficulties in making accurate determinations of adrenaline in biological fluids the results reported in the literature on the release of adrenaline during anoxia have not given absolute values for the changes. It is, however, interesting to note that in each report a relative increase of adrenal medullary activity has claimed to be demonstrated regardless of the kind of method employed (KELLAWAY 1919, HOUSSAY and MOLINELLI 1926, CRESS, CLARE and GELLHORN 1943, EMERSON and VAN LIERE 1938, RAAB 1943, LEHMANN and MICHAELIS 1942).

VAN LOO, SURTSHIN and KATZ (1948) studying the pressor responses in anoxemia concluded that the adrenals come into play in conditions of anoxemia to help neutralize the ill effects of these states by raising the blood pressure and by redistributing the blood to vital organs.

The possibility must therefore be considered that anoxic anoxia is associated with an increased adrenaline discharge. This fact bears an important significance to the present study.

Effect of adrenaline on pancreatic secretion

It has been first shown by BENEDICENTI (1906) that the pancreatic secretion elicited by secretin injection is greatly inhibited by subcutaneous administration of adrenaline. This finding has been confirmed by the work done by PEMBERTON and SWEET (1908) as well as by EDMUNDS (1910—11). The former investigators thought that the action was specific for adrenaline while EDMUNDS demonstrated that practically all substances which raise the blood pressure or stimulate the splanchnic nerves show the same effect on the pancreatic secretion. They concluded, therefore, that the anemia of this organ after the vasoconstrictor effect of such stimuli was the cause of the depressed secretion.

In the same studies Edmunds made the very interesting observation that the pancreatic vessels were extremely sensitive to adrenaline and the constriction of these vessels continued longer than in other parts of the body.

This latter part of the work has been confirmed by MANN and McLACHLIN (1917). The previous experiment described above was done with rather high doses of adrenaline without the knowledge of the dualistic action of adrenaline on the blood pressure. MANN and his associate studied the effect of adrenaline on the pancreatic secretion with large and small doses. Their conclusions were that regardless of the effect of adrenaline on the systemic blood pressure, the effect on the pancreas was always the same, namely a suppression of the pancreatic flow. The experiments were done under continuous secretin stimulation of the pancreas.

From these observations we learn that the pancreatic blood vessels are sensitive to adrenaline, that the action of adrenaline on these vessels continues longer than the changes in the general blood pressure, and that the action of adrenaline, independently of the pressor or depressor action on the blood pressure, is always the same on the pancreas, namely vasoconstriction of vessels supplying the gland. This claim leads to another important problem, to the relationship of the pancreas to an adequate blood supply.

Changes in blood supply

The work discussed on the effect of adrenaline on the pancreatic secretion already suggests that the maintenance of a normal function of this gland is closely related to an adequate blood supply.

Other experimental evidence is also at hand to elucidate these relationships. It has been demonstrated that even a slight artificial constriction of the vessels supporting the gland inhibits and occasionally stops the secretion (PAVLOW 1893, MAY 1904, ANREP 1916).

In this respect there seems to be a difference among the sensitivity of various digestive glands towards a decreased blood supply. As shown by HEIDENHAIN (1878), LANGLEY and FLETCHER (1888), CARLSON, GREER and BECHT (1907) as well as by GESELL (1920) the salivary glands are not so greatly affected by a moderate decrease in the blood supply.

On the other hand, conditions which have the tendency to increase the blood flow or amount of blood in the abdominal areas seem to be favourable for the pancreatic secretion (BABKIN 1924). Also according to PAVLOV (1893) and MAY (1904) the pancreatic gland continues to secrete some time after the blood pressure falls to zero. Such conditions are generally accompanied by a vasodilation of the abdominal vessels.

Information regarding the distribution of blood flow in various parts of the body under anoxic conditions would according to referred data be of importance in order to establish whether the consequences of oxygen lack are equally divided over the whole organism, and by this way also to discover whether the suppression of the pancreatic secretion by anoxia as it was found in the present studies could be partly explained also by such circulatory arrangements.

Although evidence has been presented that vital functions of the heart (MARKWALDER and STARLING 1913, HILTON and EICHHOLZ 1925, JOCHIM 1940, as well as GREEN and WEGRIA 1941) and central nervous system (SCHMIDT 1928, WOLFF and LENNOX 1930, LENNOX and GIBBS 1932, SCHMIDT and PIERSON 1934, GIBBS, GIBBS and LENNOX 1935, COURTICE

1941, SCHNEIDER 1942, FELDMAN, RODBARD and KATZ 1948) have preference to the blood supply during anoxia it remains to be proved that the blood is shunted from the splanchnic area to the more vital areas as has been suggested (WRIGHT 1937).

With the present knowledge no determined answer can be given whether the observed suppression of the pancreatic secretion during anoxia is brought about by disturbed blood supply of the gland under the studied conditions. Assuming such mechanism to be present, it would exert its action in two ways, first by supplying the gland with inadequate quantities of blood and secondly by allowing smaller amounts of the applied humoral stimulus, secretin, to be carried to the gland.

Changes in blood sugar

There is evidence of a considerable variety that the concentration of glucose in the blood is also concerned in the regulation of the exocrine pancreatic function.

However, the results obtained by various laboratories have not been able to bring opinions to agreement as to the exact nature of this relationship.

Much of the discrepancy might have arisen from the diverging experimental conditions used, apart from the investigators having taken into account the probable species differences in the interpretations.

It has been well established that insulin produced hypoglycemia is a powerful stimulant for the gastric secretion, this activity being carried through the vagus center to the secreting units in the stomach (ROHOLM 1930, IVY 1930, LA BARRE and de CESPEDES 1931, WELIN 1936.)

As to the pancreas, claims have been made that hyperglycemia is accompanied by a decrease in volume and enzyme output in man (OKADA and co-workers 1929, SCOTT, COLLIGNON, BUGEL and JOHNSON 1941, LAGERLÖF 1942), and that hypoglycemia evoked by insulin resulted in an increased enzyme and volume output of the pancreas during secretin stimulus (DEUSCH and DROST 1927, OKADA and co-workers 1929, FRISK and WEIN 1937, LAGERLÖF and WELIN 1937). The latter investigators were able to demonstrate a 100—200 per cent increase in the amylase and trypsin output. They failed, however, to obtain the same effect if the gastric contents were continuously aspirated during the test. OKADA and co-workers using also a double tube technique and also including achylic subjects in their material, concluded from their experiments that the increased HCl-secretion by the stomach was not responsible for the observed increase in the pancreatic secretion.

LAGERLÖF, in another series of experiments, concluded that insulin as such without the concomitant hypoglycemic effect had no influence on the pancreatic secretion.

An entirely different view has been held by BABKIN and the investigators in his laboratories (HEBB 1937, BAXTER 1932) as well as DESTREE (1930), who all claim that hypoglycemia results in a decreased pancreatic output which effect could be restored by intravenous glucose administration and that hyperglycemia was accompanied by an increased output of enzymes.

It would appear from these variations in the opinions reported, that more work is needed to settle the problem satisfactorily. In view of the fact that the pancreatic glycogen storages are depleted during secretory activity (HEBB 1938) and also that during this activity there is a rise in the blood arriving from the gland in its lactic acid contents (STILL, BENNETT and SCOTT 1933) it would perhaps be conceivable to assume that the energy for the secretory processes might be derived from the breakdown of the energy-rich carbohydrates and that the change in the availability of these substances could affect these processes. Equally justified would appear to be the claims that the hypoglycemic stimulatory effects on the vagus centres could be carried through the vagi and affect the enzyme output of the gland. As far as this work is concerned, it might be safe to point out that whatever the effects of the changes in the blood sugar levels are, these affects make their appearance on the pancreatic secretion only when pronounced. Slight fluctuations have not been shown to alter significantly this secretion.

As to the changes in blood sugar during anoxia the reported literature again offers a considerable amount of discrepancy. This was one of the reasons for including the few blood sugar determinations in the records in order to find out the magnitude of the possible changes under the conditions. As was shown, although there is a slight increase in all of the subjects, this increase is of such magnitude that, in the light of the reported literature it bears no significant relationship to the pancreatic secretion as such.

The change in the blood sugar concentration would therefore not constitute a very important causative factor in the suppression of the pancreatic secretion under the presented experimental conditions.

Summary

The effect of anoxic anoxia on the external pancreatic secretion was studied on 10 human subjects.

The pancreatic function test was performed by means of a single injection of a calculated dose of secretin (Pancreotest, Astra, Sweden) and the duodenal drainage was performed by means of a double tube technique.

The anoxic effect was produced by exposure to a reduced atmospheric pressure in a low pressure chamber. Two degrees of anoxia were employed, one corresponding to a simulated altitude of 5500 meters and the other corresponding to the altitude of 3500—4000 meters.

It was found that under the anoxic conditions the pancreatic secretion was greatly suppressed, the reduction amounting to about half of the control values. The percentage reduction was found to be greater at the higher level of simulated altitude. The reduction comprised both the volume, bicarbonate and enzyme output of the organ. From the latter function, the amylase and trypsin output were studied.

In order to elucidate the effect of concomitant alkalosis on the results, a series of experiments were performed in which 5 subjects ingested each 36 grams of sodium bicarbonate prior to the secretin test. The effect of the drug was followed by simultaneous alveolar air carbon dioxide analyses and urinary pH determinations. The pancreatic function test was performed during the highest peak of the effect of the alkalinizing salt.

It was found that the volume and bicarbonate output were slightly increased under these conditions but that the enzyme output was slightly reduced from the corresponding values observed in the control experiments. The reduction in the enzyme output was in no case of the magnitude as during the anoxia conditions.

Another series of experiments were performed on 13 dogs in order to study more accurately the details of the secretory response of the pancreas during and after the exposure to anoxia.

These studies were performed under sodium pentobarbital anesthesia by direct analyses on the juice collected from the cannulated pancreatic duct. The accessory ducts, if present, were ligated and any stimulus additional to the one given to the glands was avoided by preventing the escape of gastric contents into the duodenum. A constant secretin-pancreozymin stimulus was applied by intravenous infusion of the hormone containing solution by means of a constant infusion pump.

The anoxia was produced by allowing the animal to breathe from a gas container (Douglas sack) a gas mixture composed of 8 per cent oxygen and 92 per cent nitrogen. The ventilation was so arranged as to conduct

the expired air out of the system. The anoxic exposure was of short duration, 13 minutes in each experiment.

It was found that the anoxia produced and tested by these means also led to a decrease in the volume, bicarbonate and amylase secreted by the gland. This reduction of volume amounted to about half of the basal level and to 75 per cent for the amylase output.

A peculiar phenomenon was, however, observed during the first minutes of anoxia. It was found that during this period the volume and bicarbonate output were increased. This initial increase, although not statistically significant, was present in majority of the experiments and was attributed to the hyperventilation associated with anoxia.

In 12 of the 13 animals the recovery of the pancreatic secretion from the suppressed levels during anoxia took place in 30—50 minutes from the beginning of the breathing of normal air again. At this time, the amylase output had not, however, reached the control level, being on the average 20 per cent below this level.

From these two series of experiments it was concluded that the external pancreatic secretion is suppressed by anoxic anoxia of a short duration under the experimental conditions employed. The observed suppression involves both the hydrelatic and ecobolic functions of the gland. Although there might be an initial increased bicarbonate secretion by the gland, the total yield of pancreatic alkali secretion constitutes a reduction.

It was also concluded that the pancreas did not participate in the regulation of the acid base balance during anoxic anoxia.

The various factors which might contribute to the anoxic suppression of the pancreatic secretion are discussed. Among these the changes in the blood sugar or acid base balance may not be important. On the other hand the lack of oxygen per se must be considered among the causative factors of anoxic suppression. In addition to this, the probable role of increased adrenaline discharge by the adrenal glands is considered. The probability of inadequacy of blood supply for the pancreas on account of redistribution of blood flow during conditions of anoxia is also discussed.

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